CONTINUED APICAL DEVELOPMENT OF PULPLESS PERMANENT TEETH FOLLOWING ENDODONTIC THERAPY

By

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Submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, Indiana University, School of Dentistry, 1968.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation for the guidance and inspiration of Dr. Ralph E. McDonald for his direction of the author's graduate program and preparation of this thesis.

The author wishes to express his gratitude to Dean Maynard K. Hine and Indiana University for the opportunity to continue his education.

The author wishes to thank Dr. Grant Van Huysen for the advice and help so freely given in the conduct of this research. His devotion to this project is gratefully acknowledged, for without his guidance this thesis would not have become a reality.

The advice and ideas of Dr. Paul Starkey contributed greatly to the preparation of this thesis. The author is indebted to Dr. Starkey for his guidance in this project and wishes to take this opportunity to thank him for his inspiration and guidance.

The author wishes to thank Dr. David Mitchell for his ideas and help in interpretation of microscopic sections for this thesis.

In addition to Drs. McDonald and Van Huysen, the other members of my graduate committee, Dr. LaForrest Garner, Dr. Richard Norman and Dr. Charles Gish, supplied guidance and assistance.

The author wishes to thank Miss Shirley Shazer for the preparation of the excellent microscopic sections for this project.

Thanks are extended to Mrs. Mildred Redford and assistants for their excellent care of the experimental animals and assistance during procedures performed on the animals. The author also wishes to thank Mrs. Isabel Poor for her assistance in processing radiographs used in this project.

Special thanks are extended to Dr. Charles Tomich for the preparation of the illustrations used in this thesis. The help of Mrs. Helen Campbell and the library staff is gratefully acknowledged.

Thanks are extended to Dr. Donald Bowers and Mrs. Sara Snyder for editing of this thesis and to Mrs. Twila Chapman for typing of the final copy.

The author wishes to take this opportunity to thank his parents for their many sacrifices and inspirations which have enabled him to complete his academic career.

Finally, my sincere thanks to my wife, Linda, for typing of this thesis and for her sacrifices, encouragements and understanding during my graduate training.

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INTRODUCTION

It is generally accepted that successful endodontic treatment requires the apex of the treated tooth be hermetically sealed with root canal filling material.[1-3] The traditional approach to treatment of the pulpless permanent tooth with an incompletely developed root having a divergent apex has been surgical.[4-6] Surgical techniques to achieve the complete apical seal have utilized gutta-percha and other materials or have entailed preparation of the apex to receive an alloy of silver amalgam.

Although the surgical approach has been successful, the mechanical and psychological aspects may offer contraindications to this approach. In the pulpless tooth with an incompletely formed apex, the thin, fragile dentinal walls make it difficult to achieve an apical seal. When a portion of the root is removed surgically in order to obtain a seal, the crown-to-root ratio is often less than desirable. The surgical approach is a traumatic experience for the young child and a technique with less trauma is desirable.

Continued apical development of the incompletely developed root apex after vital pulpotomy procedures is well documented in the literature.[7, 8] Techniques reported in recent investigations[9-12] demonstrated that apical development may continue in the pulpless permanent tooth after the necrotic contents of the root canal are removed and a dressing placed in the tooth for a period of six to nine months. Subsequent to the apical

development, the canal may be sealed in the routine manner with a primary gutta-percha cone and lateral condensation without having to resort to the surgical procedures mentioned previously.

This study was designed to investigate the non-surgical treatment of the pulpless permanent tooth with a divergent apex. Apical and periapical changes following treatment will be investigated histologically.

REVIEW OF THE LITERATURE

The following review of the literature is confined to the four spheres of research which provide the necessary background to this study. The first review describes Hertwig's epithelial root sheath and normal root formation. Second is a description of tissue reactions to camphorated parachlorophenol (CMCP) when placed in root canals and when implanted in the connective tissue of animals. The third section examines the use of calcium hydroxide as a root canal filling material and the connective tissue responses of various animals to implanted calcium hydroxide. Fourth is a review of the literature dealing with the non-surgical treatment of pulpless permanent teeth with incompletely formed apices.

HERTWIG'S EPITHELIAL ROOT SHEATH AND ROOT FORMATION

Many authors[13-18] describe in detail root formation of the tooth. According to Orban,[13] development of the roots begins after enamel and dentin formation has reached the future cemento-enamel junction. The epithelial dental organ forms Hertwig's epithelial root sheath which initiates formation and molds the shape of the roots. Hertwig's epithelial root sheath consists of the outer and inner dental epithelium without a stratum intermedium or stellate reticulum. The cells of the inner layer of Hertwig's epithelial root sheath remain short and normally do not produce enamel. Once these cells have induced the differen-

tiation of connective tissue into odontoblasts and the first layer of dentin has been laid down, the epithelial root sheath loses its continuity. Remnants of the root sheath persist as epithelial rests of Malassez.

Hertwig[19] first suggested the term "epithelial sheath" in 1874. He stated, "The lower part of the epithelial mantle can in this instance no longer be designated as the enamel membrane, but one must instead, differentiate it with the term epithelial sheath." This observation was made from the histologic study of an amphibian's tooth. Hertwig concluded, "By an increase in the length of the epithelial sheath in a downward direction, the tooth outline is lengthened, and the germ tissue as a result assumes the form of the future root."

According to Orban,[13] Hertwig's epithelial root sheath is formed from cells in the cervical loop. The cervical loop is the free border of the dental organ where the inner and outer dental epithelial layers are continuous and reflected into one another. When the dental organ of the crown is formed, the cells of the cervical loop portion give rise to Hertwig's epithelial root sheath.

Schour[14] states that prior to the beginning of root formation, and at the level of the cemento-enamel junction, Hertwig's sheath bends at a right angle to form the epithelial diaphragm. Hertwig's sheath takes the form of one or more epithelial tubes, depending on the number of roots of the tooth. In a single

rooted tooth, the epithelial sheath is a single tubular structure. In a multi-rooted tooth, the cervical opening is bridged by horizontal flaps of the diaphragm forming a tubular structure for each root. In the root, with respect to dentin formation, the epithelial sheath continues the function of the odontogenic organ.

According to Diamond and Applebaum,[20] the formation of dentin within the crown is initially activated by the ameloblasts, whereas the dentin formation of the root is initially activated by Hertwig's epithelial root sheath. This finding was based upon a study of human teeth in the early formative stages of development. They demonstrated that the epithelial sheath is a continuation of the outer and inner enamel organ epithelium. According to their findings. the sheath is not manifested until the formation of the enamel matrix of the crown is appreciably advanced. At this stage the epithelial sheath is outlining the contour of the future root and remains in an undifferentiated state which is activated only after all the enamel matrix is formed and calcification has started. The lack of differentiation is evidenced by the absence of odontoblastic formation of the adjacent connective tissue of the dental papilla.

Logan[21] studied the development of the epithelial root sheath in the first molars of 24 Sherman albino rats. Serial sections of the first molar teeth of the rats aged two to 45 days were prepared. Root formation began after development of the dentin eminence, which forms the cervix of the rat molar. The epithelial

root sheath was shown to develop from a growth center at the fundus of invaginating primordial ectoderm. Daughter cells of the fundic growth center gave rise to the epithelial diaphragm. The epithelial root sheath was the next cytomorphic stage of radicular dental epithelium development. The root sheath was shown to be a double layer of cells adjacent to newly formed predentin. The sheath was continuous with the enamel organ elements only at the start of root formation.

According to Orban,[13] advancing age induces a decrease in the size of the root canal. The apical foramen has a wide opening, limited by the epithelial diaphragm, during root formation. The dentinal walls taper apically and the shape of the pulp canal is like a wide, open tube. As growth proceeds, the root canal is narrowed by the formation of more dentin.

The relationship of Hertwig's epithelial root sheath to cementogenesis has not been clearly established. Schour and Massler[22, 23] believe that the root sheath probably stimulates the cells of the dental follicle to form cementum after which they degenerate and their remnants can be found in the periodontal membrane as epithelial rests. On the other hand, Orban[24] believes that following the initiation of cementogenesis, the epithelial sheath becomes separated from the root and continues to exert a stimulating influence on the cementoblasts as epithelial rests. The epithelium remains viable within the periodontal membrane as the epithelial rests of Malassez. The presence of

these epithelial remnants within the periodontal membrane was first described by Malassez[25] in 1884.

Orban[13] reported that as root formation progresses apically, Hertwig's epithelial root sheath breaks up into epithelial rests and cementum is deposited on the root surface. The continuity of the sheath is broken either by partial degeneration of the epithelium or by active proliferation of connective tissue of the dental sac. When contact of the periodontal connective tissue is established with the root surface, cementoblasts are formed from the mesenchymal connective tissue elements. These cementoblasts produce cementum in two consecutive phases. Uncalcified cementoid tissue is laid down, and in the second phase, the cementoid tissue is transformed into calcified cementum. A thin layer of cementoid type tissue is always seen on the surface of the cementum, since growth of the cementum is a continuous process. The cementum formation influences the size and shape of the apical foramen in the fully formed tooth. Root canals are not always single and straight, and may vary by the occurrence of accessory canals.

Diab and Stallard[26] reporting in 1967, showed a definite similarity in the analysis of the life cycles of cells of the cervical loop of the enamel organ and the lower portions of Hertwig's epithelial root sheath. They conducted a radioautographic study into the life cycle and migration of cellular elements of the developing roots of first molar teeth of 54 Sprague-Dawley rats. The animals were injected with tritiated thymidine and the tissues

processed histologically for radioautographic analysis. It was demonstrated that the sheath cells appeared to degenerate within the periodontal membrane, and therefore did not contribute to the formation of the epithelial rests of Malassez. Six days after injection, labeling was not demonstrated by any cells of the root sheath except those trapped during cementum formation. The remaining sheath cells appeared to have degenerated. Diab and Stallard concluded that an interdependence between odontoblastic differentiation and cellular activity of the epithelial root sheath was demonstrated. Cementum formation appeared dependent on neither the presence nor the absence of the epithelial root sheath.

Bhaskar[16] and Orban[13] explain the formation of accessory foramina as a defect in Hertwig's epithelial root sheath around a large blood vessel or nerve entering the dental papilla. Since odontoblasts differentiate only where Hertwig's sheath is present, the area of the blood vessel or nerve remains as a defect in the dentinal wall of the root.

Recent transplantation research by Hoffman[27] demonstrated experimentally that the developing root exerts its influence even on connective tissue of non-oral sites. Molar tooth germs were removed from the dental follicles in hamsters' jaws before root formation began and transplanted into the connective tissue under the skin of the back. These transplanted tooth germs succeeded in organizing new dental follicles, apparently from

the connective tissue of the skin. The teeth were noted to undergo root formation. They also induced the surrounding connective tissue to form cementum, periodontal membrane and alveolar bone. Based upon his experiment, Hoffman concluded that the source of stimulus for formation of the periodontal tissues came from the transplanted tissues. He proposed that the most likely source of the stimulus was the enamel organ and its derivative, Hertwig's epithelial root sheath.

According to Permar,[28] root length is not completed until one to four years after a tooth erupts into the oral cavity. A newly emerged tooth has a short root and a very large apical foramen. Gaunt, Osborn and Ten Cate[18] reported that the length of the root is a function of either the degree of intrinsic growth of Hertwig's epithelial root sheath or of growth of the tooth papilla. It has not been conclusively established which tissue plays the dominant role.

TISSUE REACTIONS TO CAMPHORATED PARACHLOROPHENOL

According to Grossman[29] chlorophenol is a substitution product of phenol with chlorine replacing one of the hydrogen atoms. It is more bactericidal than phenol and much less caustic. When trituated with gum camphor, it combines to form camphorated parachlorophenol (CMCP). The disinfectant action is due to the slow liberation of chlorine in the presence of phenol. The camphor

reduces the irritational and caustic properties while serving as a diluent and vehicle.

Walkhoff[30] introduced chlorophenol as a root canal disinfectant in 1884. He stated that chlorophenol had the property of almost immediate relief of toothache with its origin in the pulp. Walkhoff felt the foremost quality of the medicament was its antiseptic power and its quick penetration of the tissues. He stated that when chlorophenol was placed on vital tissues, it saturated the tissue only to a certain degree, without cauterizing the entire pulp.

In 1932, Coolidge[31] removed the vital pulps from dogs' teeth and sealed drugs in the canals for 21 days. Phenol, cresol, creosote and cresatin were the medicaments tested. He found some drugs more irritating than others. He attributed the irritation to the properties of penetration. Those drugs which coagulate albumin appear to be self-limiting in their actions and did not penetrate deeply in periapical tissues. Therefore, less destruction of living tissue was found about the root apices when those drugs were used.

Coolidge believed that drugs which were germicidal, but not self-limiting by the coagulation of protein, were harmful to living tissue. The action of such drugs is continuous until the drug itself becomes sufficiently combined or diluted to stop its action. However, these penetrating drugs were judged more efficient for sterilization of inaccessible portions of infected root

canals. He also pointed out that irritation from the drug itself might be less than the irritation caused by bacteria remaining in the canal and gaining access to the periapical tissues.

In 1932, Grossman and Prinz[32] conducted a clinical evaluation of CMCP. The medicament was compared to electro-sterilization in a study of the sterility of root canals determined by smear and culture techniques. In a group of 60 patients, it was concluded that the electro-sterilization technique was more efficient in achieving sterility than the method of sealing a medicament such as CMCP in the root canal of a tooth. An average of five treatments, in a period of 23 days, was required to obtain negative cultures in the teeth treated with CMCP.

Pear[33] compared the bactericidal effect of various medications used to sterilize root canals. In this bacteriologic study the medication was placed on pieces of paper in the centers of agar and blood-agar plates which had been inoculated with Staphylococcus aureus. The culture media was then incubated. A comparison of the inhibition of growth around the medicaments was made. Formocresol and CMCP were proven to be the most effective. Pear stated that CMCP was the drug of choice because of its non-irritating qualities.

Ostrander, Crowley and Dowson[34] compared the effectiveness of commonly used root canal antiseptics on the basis of the number of teeth becoming sterile after one treatment. CMCP was shown to be more effective than penicillin, eugenol or formaldehyde-

cresol solutions. All cases were treated with the same technique except different drugs being sealed in the canals.

Ostrander and Crowley[35] found CMCP to be as effective, or in most instances more effective than other more highly caustic agents used to sterilize root canals. In this clinical evaluation, cultures were taken of routine cases being treated to determine the amount of time required to achieve two negative cultures.

Ostrander[36] reporting in 1958, again advocated the use of CMCP as the root canal medicament of choice. He pointed out that the agent was highly effective and virtually non-irritating under the conditions of use advocated. Data was presented from five different sources, two private offices and three years of clinical study at the University of Michigan Dental Clinic, and involving completely different sets of cases. The medicament was shown to be consistently effective. The average number of treatments required to obtain two negative cultures was from 3.47 to 4.2 treatments. Ostrander compared the effectiveness of other medicaments, including the antibiotics, to CMCP. No other medicament was shown to be more effective than CMCP.

In 1958, Ingle and Zeldow[37] reported a clinical-laboratory evaluation of three intra-canal antibacterial agents. The three drugs used in the study were CMCP and two polyantibiotic mixtures. Upon entry into the canals, cultures were taken of cases to be treated in the undergraduate endodontic clinic at the University of Washington. By these initial cultures the bacteriologically

negative cases were eliminated from the study. The canals were instrumented, irrigated and dried in the usual manner. The medications were assigned as unknowns, placed in the canals and sealed with cement. The root canal was treated until one negative culture was obtained. The results showed no statistically significant difference in the effectiveness of the three medicaments tested. Ingle and Zeldow concluded that at present they could not recommend one drug over the other.

Sommer, Ostrander and Crowley[38] compared the effectiveness of CMCP with other medicaments presently used in sterilization of root canals. Their studies have shown CMCP to be more effective than the older caustic drugs. The amount of time required to obtain two negative cultures was similar to that required when using polyantibiotic mixtures. As a supplemental medicament, the authors advocate the use of a mixture of CMCP and penicillin. The two drugs are compatible and together form a mixture which is effective against about all types of organisms found in the root canal.

Several studies of the irritational potential of root canal medicaments have been reported. The earliest reported investigation including CMCP was by Grossman[39] in 1944. Cotton pellets containing root canal medicaments were placed in contact with the shaven forearm and covered. The medicaments tested were azochloramid in triacetin, beechwood creosote, CMCP, cresatin and formocresol. After 48 hours the dressings were removed and photographs

were taken of the areas. Grossman noted no objective signs of irritation or inflammation present where azochloramid, CMCP or cresatin had been applied. No subjective symptoms were noted by the patients except slightly irritating itching. Severe or mild areas of inflammation were noted with beechwood creosote and formocresol. The areas were painful. From this study, Grossman concluded that CMCP was non-irritating to tissues and the medicament was safe to use in root canals.

In 1958, Rubbo, Reich and Dixson[40] studied the reactions of the subcutaneous tissue of rabbits to root canal medicaments. They injected 0.1 ml. of the solutions subcutaneoulsy into rabbits' ears. Effects were evaluated by the degree of inflammation, necrosis and ulceration after 24 hours, three days and seven days. Reactions were evaluated macroscopically and microscopically. They showed that CMCP, beechwood creosote, formocresol and oxpara liquid induced severe necrosis with ulceration.

Schilder and Amsterdam,[41] in 1959, used rabbits to investigate the inflammatory potential of endodontic root canal medicaments. They injected 0.1 c.c. of each drug intradermally into the abdomen of the animals. This was followed by an intravenous injection of the vital dye, trypan blue. The accumulation of trypan blue around the implant site was the criteria for judging the response to the injected medicaments. Theoretically the dye should accumulate in concentrations directly proportional to the amount of capillary permeability present, which in turn is an

indicator of the inflammatory response. Macroscopic observations one day succeeding the injections demonstrated a severe inflammatory reaction around beechwood creosote, azochloramid, CMCP, eugenol, formocresol and chlorinated soda. According to the authors the significance of the histologic slides was limited. In a second phase of the investigation, Schilder and Amsterdam introduced 0.15 c.c. of each drug into the conjunctival sac of the eyes of rabbits. Physiologic saline solution was used as a control in the experiment. Using the criteria of swelling and hyperemia of the conjunctiva, clouding of the cornea, loss of clarity of normal anatomic structures, external swelling and exudation, CMCP was again shown to cause a severe inflammatory response.

In 1960, Grossman[42] reported a study in which he injected root canal medicaments into the abdominal connective tissue of guinea pigs. He found that cresatin, CMCP, beechwood creosote and PBSC antibiotic mixture irritated the tissue.

In 1961, Torneck[43] evaluated the inflammatory potential of 10 root canal medicaments using the subcutaneous tissues of hamsters. Punctured polyethylene carpules containing the medicaments were implanted surgically into the dorsal, subdermal tissues of the hamster. Physiologic saline solution was used a control. Histologic sections were prepared following test periods of 48 and 96 hours. The inflammatory response to CMCP was judged to be moderate at both test periods. No evidence of tissue necrosis was evident about the implant sites. In the 96 hour specimens

an active condensation of young connective tissue was noted about the periphery of the carpule away from the site of the opening. Torneck concluded that the reactions encountered clinically with the use of root canal medicaments are influenced by many factors. The type, concentration and physical form of the drug utilized are important factors. The volume of drug, the manner in which the drug is sealed into the root canal and the length of time the drug is exposed to the tissues effects the reaction of the tissues to the medicament. Other important factors are the size of the apical foramen, the histologic status of the periodontium and the susceptibility of the individual to injury. A higher incidence of pericementitis and a reduced rate of healing are found with drugs which are highly irritating.

Arefian,[44] in 1962, reported on the vital tissue tolerance of hamsters to various root canal medicaments. He injected 0.1 c.c. of the medicaments in the connective tissues of the abdomen, pelvic and shoulder areas. Test periods included six hours, 48 hours, six days, 16 days and 32 days. An immediate discoloration of the area was noted as the only gross observation change during the test period. An arbitrary classification of mild, moderate or severe was used to classify the inflammatory response. The inflammatory response was judged according to types and number of leukocytes, amount of edema, fibroblastic activity and fibrosis, degree of vascularity, muscle degeneration, necrosis and amount of tissue destruction. The six hour response

to CMCP was slight edema with polymorphonuclear leukocytes. Hyperemia, rupture of blood vessels with extravasation, and some muscle fiber degeneration was also noted. In the 48 hour sections, Arefian found a moderate number of polymorphonuclear leukocytes with a few lymphocytes in a slightly edematous intermuscular area. Cellular infiltration into the dermis and muscle bundles caused partial degeneration of the muscle. Wide areas of fibrosis were noted. After six days, he found very few inflammatory cells. There was degeneration of some muscle fibers with replacement by fibrous tissue. At the 16 day period, Arefian noted no inflammatory cells present. Granulation tissue had replaced some muscle fibers which had undergone degeneration. The degree of fibrosis was classified as mild. In the 32 day sections, he found all areas healed and normal. Arefian classified the overall reaction of the tissue to CMCP as a mild reaction.

CALCIUM HYDROXIDE AS A ROOT CANAL FILLING MATERIAL

Hermann[45] introduced the use of calcium hydroxide in pulp capping in 1930. Since its introduction the role of calcium hydroxide in pulp capping and the formation of secondary dentin has been widely investigated.[46-49]

In 1938, Teuscher and Zander[50] reported the action of calcium hydroxide when used in pulpotomies. The formation of a dentin-like material above a layer of odontoblasts just beneath the exposure site was noted in this histologic report. This calcified mass

was referred to as a dentin bridge which corresponded to the radiographic appearance of a radio-opaque line beneath the medication.

The reader is referred to Compton[51] for a complete review of the literature on the use of calcium hydroxide in vital pulp capping and pulpotomy procedures.

According to Laws,[52] the material which is most consistently associated with calcified repair tissue is calcium hydroxide. Despite this advantage, little has been reported of its use following pulp extirpation.

The first reported use of calcium hydroxide in filling root canals was by Rohner[53] in 1940. Twenty teeth were filled with Calxyl (calcium hydroxide in Ringer's solution) for periods of four to 11 months. Although the study contained too many variables to determine the actions of the medication, it is significant that cementum-like tissue was described as sealing the apices of several of the teeth.

Castagnola and Orlay[54] advocated the filling of root canals with iodoform paste. They advised the use of Calxyl as an alternative filling material in cases with iodine allergy or in cases of rare osteosclerotic conditions. In the patient with persistent pain in a treated tooth, where no reason can be found for the discomfort, they suggest removal of the filling material and placement of Calxyl. Success will frequently occur in these cases. The

reason speculated by the authors is the constant alkaline influence of the Calxyl. No further details of their technique or their results are given.

In 1958, Matsumiya and Suzuki[55] presented the results of 24 years of studies on root canal treatment. Over this period of time, 74 operators were involved. Histopathological observations were made on the effects of 120 kinds of filling materials, 30 kinds of disinfectants and other drugs on about 18,000 teeth of 660 dogs and 602 teeth from 586 clinical patients. From the tests it was determined that the best filling materials were a "calcium hydroxide paste and a few other pastes composed chiefly of 1-2% paraform powder." Matsumiya and Suzuki found these pastes to promote proliferation and cicatrization of the granulation tissues in the periapical region. Accelerated proliferation of the cementoid tissue necessary for closure of the apical foramen as well as active regeneration of the alveolar bone destroyed by inflammation was noted when these pastes were used to fill root canals. They advocate the perforation of the apical foramen during the cleansing of the canals in order to remove the most infected dentin at the wall of the root canal and any necrotic tissue attached to the dentinal wall. Secondly, perforation allows for the removal of gases or exudate present in the periapical tissues. Perforation also establishes a route for the filling material to extend its biological influences into the periapical tissues.

Matsumiya and Suzuki classified the completely healed conditions resulting from the root canal treatment into five basic types: (1) the formation of a cicatrized root canal polyp; (2) the closure of the apical foramen by the proliferation of hard tissues; (3) the periapical focus of infection is healed by the formation of healthy fibrous connective tissue, though regeneration of the destroyed alveolar bone is hardly observable radiographically; (4) encapsulation of filling material forced through the apical foramen by a thick layer of healthy fibrous connective tissue, or the proliferation of cementoid tissue; and (5) the active regeneration of the alveolar bone.

Matsumiya, Suzuki and Takuma,[56] in 1962, presented histologic sections of human teeth and dogs' teeth which demonstrated the five types of healing previously described. Calcified material was demonstrated forming in the connective tissue at the apices after filling of canals with "calcium hydroxide paste." The results were shown when treating uninfected as well as infected root canals.

In 1960, Machida[57] reported a study in which calcium hydroxide mixed with prophylene glycol was used to fill root canals. Of the 50 teeth used in the study, 25 teeth were subjected to vital pulp extirpation. The remaining 25 teeth were devitalized with "Neo-arsen black" and the pulps were then extirpated. The root canals were filled with the drug and the teeth were evaluated "clinico-pathologically" from two to 279 days. The healing process

after placing the root canal fillings was classified as two types: healing originating in vital pulp remnants, and healing originating in the periodontal membrane. Of 21 teeth showing healing in vital pulp tissue, new dentin barriers were found in seven instances of the vital pulp extirpation and in three of the teeth in the devitalized group. Among 26 teeth showing healing originating in the periodontal membrane, 10 of these were of the vital extirpation group and 16 were of the devitalized group. In each of these 26 teeth, cementum and dentin near the apical foramen had been resorbed and new cementum deposited upon the resorbed surfaces. An evaluation of the response of the periapical tissues was not included in the report.

In 1961, Seltzer, Bender and Kaufman[58] reported the findings of a study on dogs to determine if apical repair could be stimulated by filling root canals with calcium hydroxide. Vital pulp extirpations were performed and the canals instrumented. Calcium hydroxide and distilled water were placed in the canals after drying with paper points. The orifices were sealed with amalgam. In one week sections, an acute inflammatory response was noted resulting from the extirpation and filling of the canal. The connective tissue was heavily infiltrated with polymorphonuclear leukocytes. A profuse edema was found within the apical periodontal membrane and the marrow spaces of the bone. There was no evidence of new matrix activity found in the tissue. In the two week sections, the acute inflammatory response was more pronounced.

The fibers of the apical periodontal membrane were separated by edema and cells. Osteoclastic activity with bone resorption was occurring at sites distant from the apical foramen. At three weeks, an acute apical dentoalveolar abscess had developed. Necrotic tissue and suppuration were present. Bone resorption had created a large space apically. In the four week sections, they found an acute apical abscess. In no instance did Seltzer and his coworkers find physiologic closure of the apical foramina by newly formed cementum or any evidence of new matrix activity.

Laws[52] reported, in 1962, that calcium hydroxide was well tolerated by the apical tissues when used as a root canal filling material. He treated eight teeth by vital pulpectomy two millimeters short of the root apex. The reason given for partial pulp extirpation was to ensure that some apical tissue would remain for histologic evaluation after the teeth were extracted. Calcium hydroxide mixed with propylene glycol was introduced into the canals and spiral root canal fillers used to work the material into the apical region. The period of observation was from 19 to 126 days. Histological examination after extraction showed resorption of the dentinal walls of the canals followed by deposition of cementum. With resorption of the filling, an ingrowth of healthy fibrous connective tissue from the periodontal membrane was observed. Calcification of the remaining pulp did not occur, nor was there any evidence of osteogenic activity.

In a very limited study, reported in 1965, Nyborg and Tullin[59] found healing around root canals filled with calcium hydroxide. Seventeen teeth with healthy pulps were subjected to pulp extirpation ranging from five millimeters from the apex to complete extirpation. Dressings of one per cent Lugol's solution followed by gutta-percha filling material or a paste of calcium hydroxide mixed with Ringer's solution were placed in the canals. Healing occurred regardless of the dressing used.

Maruzabel and her associates,[60] in 1966, reported a study of periapical overfilling of the root canals of rat molars. They used resorbable pastes of iodoform and occasionally the addition of zinc oxide or calcium hydroxide. When forced through the apices of molars which had been mechanically cleansed, the materials were rapidly resorbed. Fragments of the pastes were rapidly surrounded by polymorphonuclear leukocytes. Several days later macrophages appeared and the over-filling material was rapidly resorbed.

In 1967, Rowe[61] reported a study in which he filled the root canals of three teeth of a cat with calcium hydroxide mixed with distilled water. Vital pulp extirpations were performed and the canals enlarged in the usual manner. The material was placed in the canals with a rotary canal filler. Rowe found a severe inflammatory response associated with abscess formation in the periapical tissues. In eight week sections, he found bone resorption. The sample was too small to draw any conclusions as to the action of the calcium hydroxide on the tissues.

TISSUE RESPONSES TO IMPLANTED CALCIUM HYDROXIDE

The effects of calcium hydroxide on connective tissue have been studied by numerous investigators by implanting pellets of calcium hydroxide in the subdermal connective tissues of various animals. Schaad, Carter and Meyers,[62] in 1958, implanted dental materials into the abdominal connective tissue of rats and examined the results after 24, 48, and 96 hour intervals. They found calcium hydroxide induced a slight inflammatory reaction. The results were correlated with their findings when the same materials were used to cap vital pulps in rat incisors. They suggest a similarity between the reaction of the abdominal connective tissue in rats and the calciotraumatic line evidenced in rat incisors. No mention was made of calcification around the implants of calcium hydroxide.

In 1957, Mitchell and Amos[63] reported a microscopic evaluation of implants in the dorsal connective tissue of rats. Calcium hydroxide was reported to have induced marked fibroplasia, mild inflammation and heterotopic bone formation. In 19 intervals ranging from two to 39 days, heterotopic bone formation was found in the enclosing fibrous capsule in all except one specimen.

Shankwalker[64] created defects in the jaws of dogs by raising a gingival flap and resecting a block of bone, dentin, and cementum with a bone bur. Sixteen defects were filled with calcium hydroxide and 16 were left unfilled to serve as controls. Although

the calcium hydroxide implants showed some osteogenic potential, there was no rapidly apparent reconstruction of the resected alveolar bone. Both implants showed a favorable effect on the regeneration of the periodontal ligament and the cementum in the implanted areas. Osteogenic formation was noted in 18 day sections (the shortest time period) but increased greatly at 43 days. New cementum was not noted until the 43 day sections. Reattachment of the fibrous connective tissue was seen taking place through the medium of the newly deposited cementum. Osteoblastic and osteoclastic activity was noted in the specimens.

Mitchell and Shankwalker[65] studied the osteogenic potential of 13 materials by the implantation technique. Materials were implanted into the dorsal connective tissue of rats for periods up to 39 days. Heterotopic ossification or calcification was reported consistently around specimen of calcium hydroxide and water, and calcium hydroxide plus methyl cellulose. An early inflammatory reaction was noted surrounding the calcium hydroxide implants. The inflammatory reaction was replaced by fibroplasia with ensuing capsulation.

Heterotopic osteoid tissue formation surrounding implants of methyl cellulose and calcium hydroxide, and Gelfoam and calcium hydroxide was reported by Mitchell[66] in 1958. Pellets of calcium hydroxide and water induced heterotopic osteoid tissue in the capsule around the material at 17 different intervals from two to 200 days. Calcium hydroxide and methyl cellulose gave similar

reactions at six intervals from three to 33 days. Calcium hydroxide and Gelfoam pellets stimulated osteoid formation after 26 days, but not after 17 days.

Zawawi[67] implanted materials in the connective tissue of rats and studied the effects at two, 16 and 32 days. Pure calcium hydroxide and the commercial preparations Serocalcium paste (calcium hydroxide with the salts $NaHCO_3$, $CaCl_2$ and KCl of human blood serum) and Hydroxyline (calcium hydroxide suspended in copolymer plastic and a solvent of methylethyl ketone) were osteogenic. The appearance of osteoid material was noted in close association with adipose tissue, while calcification was observed within the adjacent subdermal muscle. Osteoid material and calcification were observed in the two-day sections. Chembar (composed of calcium hydroxide, zinc oxide, polystyrene and chloroform) and commercial preparations of zinc oxide-eugenol did not induce osteogenesis. Serocalcium and Chembar produced severe inflammatory reactions, while Hydroxyline and calcium hydroxide elicited a moderate response. Zawawi correlated the formation of dentin bridges over pulp exposures capped with calcium hydroxide to the findings in her study of formation of osteoid material within the connective tissue of rats. Zawawi also correlated her findings with Chembar with those of Zander and associates,[68] who showed no secondary dentin formation under pulps capped with Chembar. She postulated that the presence of other ingredients in Chembar resulted in the non-osteogenic effect of the material.

Traicoff,[69] in 1958, implanted calcium hydroxide and sodium fluoride in the dorsal connective tissue of scorbutic and normal guinea pigs. Sections at three, eight and 35 days showed a moderate connective tissue reaction and osteoid formation. Less osteoid formation was noted in the scorbutic than the normal guinea pig.

Mitchell,[70] in 1959, published another report of implant studies in the subdermal connective tissue of rats. After periods of two days, two weeks and four weeks the tissues about the implanted materials were studied microscopically. The inflammatory reactions to the materials were classified as mild, moderate or severe. Mitchell advocated this simple, short-term screening test to determine the relative irritational qualities of materials used in dentistry. Tissue reactions about calcium hydroxide mixed with water implants were consistently moderate in nature at the three intervals. A zone of coagulation necrosis immediately surrounding the material was present in the four day specimens. A thick inflammatory and fibrous capsule was also present. Some pathologic calcification of the subdermal muscle bundles was noticed. In the 16 day specimens a thick fibrous capsule was present. Considerable osteoid tissue was deposited within the capsule in close association with fat tissue. Four week specimens revealed a continuation of the moderate inflammatory reaction and the presence of osteoid and associated giant cells. When Mitchell added calcium hydroxide to a zinc oxide-eugenol and zinc acetate mixture,

a moderate persisting inflammatory reaction resulted. The reaction was comparable to that of calcium hydroxide alone; however, no stimulation of osteogenesis was present.

In a study of the tumorigenecity of dental materials, Mitchell, Shankwalker and Shazer[71] implanted 12 dental materials in the subdermal connective tissue of rats. No evidence of tumor formation was noted about implants of calcium hydroxide.

Yoshiki and Mori[72] studied the tissue reaction to calcium hydroxide from the standpoint of enzyme histochemistry. Small amounts of calcium hydroxide paste were implanted in the dorsal subcutaneous tissue of rats and guinea pigs. Calcified tissue formation was found in one to four week sections after the implantation. A histochemical survey of enzymes present in the tissue surrounding the implants revealed similar enzymes to those associated with normal calcification.

In 1966, Ronning and Koski[73] contradicted some of the findings of Mitchell. In a study of the osteogenic potential of implanted lumps of dehydrated calcium hydroxide and anorganic bone soaked in calcium hydroxide, no osteoid formation was noted. The materials were implanted in the subcutaneous tissues of rats, and sections were made at two, four, eight, 16, 32, 64, and 96 day intervals. The inflammatory response of the tissues was not evaluated; however, it was noted that several of the implants of calcium hydroxide extruded through the skin of the rats.

In 1967, Binnie[74] implanted calcium hydroxide and other dental materials in the subdermal tissue of rats in a histochemical study of induced calcification. He used more sophisticated methods of study than those used in previous studies. These included the use of the fluorescent stains, tetracycline and Procion red dye, and a histologic observation of enzyme activity in the tissues. Histochemical methods were utilized to study the nature of the tissue reaction to these materials and the substance produced by the tissues if any resulted. Severe acute inflammatory reactions and some necrosis, particularly of fat, were present around the implants initially. However, after eight days the inflammatory reaction was almost totally chronic in nature. Cellular connective tissue capsules around the implants contained small globular areas of bone. After 16 days the inflammatory reactions had given way to repair.

Binnie attempted to identify the calcified material formed in the tissues adjacent to the implanted calcium hydroxide. He showed that the osseous product contained PAS positive and alcion blue positive material, which are necessary criteria for bone matrix, but which are not themselves diagnostic. Tetracycline fluorescence was observed in the osseous product, but in none of the other tissues or materials. There were no recognizable osteoblasts or any evidence of pre-mineralized bone matrix. The crystal structure of the mineral product was unknown. The matrix appeared to be a degenerated or necrotic collagen and lipid structure similar histochemically to bone matrix, but without the

normally associated cells. Binnie classified the osseous tissue as an amorphous, lamellar, non-cellular hard tissue similar to some forms of dystrophic bone.

In 1967, Prescott[75] investigated the connective tissue response of dogs and monkeys to dental materials implanted under strict aseptic conditions. He felt that since most implant studies had been carried out on rodents that the responses of the dog and monkey would be closer to man phylogenetically. Histopathologic sections were taken at four, 15 and 30 day intervals. In the dog at the 15 day interval a severe inflammatory reaction existed around implants of calcium hydroxide. The severe reaction was persistent at the 30 day interval. Osteoid formation was noted in the 30 day sections. In the monkey in 15 day sections the inflammatory reaction was judged to be severe. The reaction remained severe in the 30 day sections. Osteoid formation was not mentioned in an evaluation of the tissue reaction in monkeys. Prescott noted that overall, the connective tissue responses in the dog and monkey were more severe than those reported in the literature for rodents. The four day responses in both dogs and monkeys were extremely difficult to evaluate due to large amounts of hemorrhage present from the surgical procedure. Therefore, Prescott made no conclusions for the tissue reactions at the four day interval.

NON-SURGICAL TREATMENT OF PULPLESS PERMANENT TEETH WITH INCOMPLETELY FORMED APICES

It has long been known that extirpation of the vital dental pulp and successful sealing of the root canal with an inert material will result in a closure of the apical foramina by cementum.[52] Davis[76] examined over 100 teeth by making ground sections of teeth which had been filled short of the apex by one to three millimeters. In these teeth, he reported that the remaining portions of the canals had been entirely filled with cementum, dentin or "calcic matter" so that no openings existed in the canals at the time of extraction. Teeth with single and multiple foramina were included in this study. In infected teeth he reported minute openings persisted even though a marked hyperplasia of the cementum had occurred.

Hatton[77] reported a histologic study of incompletely filled teeth which were later extracted. Complete or nearly complete closure of the incompletely filled canals at the apical portion was observed in many sections. The structure of the plug of calcified material more often resembled cementum than dentin, or it was quite amorphous or lamellated.

Grove[78] demonstrated the deposition of cementum within the root canal near the apex of an incompletely formed tooth which had the pulp removed three months previous to extraction. Histologically, he showed a concentric layer of cementum inside the root canal near the apex. Grove stated that when all the pulp tissue

is removed from a root canal that a fibrous plug develops. Cementoblasts are formed and cementum is deposited at the apex.

In 1929, Applebaum[79] reported two teeth in which there was pulpal necrosis while the apices were divergent. Neither of these teeth were treated; however, there was a marked effort to prevent bacterial invasion of the surrounding tissues. The formation of a calcified plug at the apex of each tooth was an attempt by the body to adapt itself to the accidental death and decomposition of the pulp by a change in the normal morphology of the tooth. Upon histologic examination the plug of calcified material was shown to be composed of osteoid cementum and a core of irregular dentin.

In 1940, Rohner[53] demonstrated six instances of closure of the apical foramen by the apposition of cementum after filling of the root canals with Calxyl (calcium hydroxide in Ringer's solution). In these cases the root canals were filled with Calxyl after vital pulp extirpations. The observation period was seven months.

In 1943, Easlick[80] reported a case of continued apical development following the treatment of an abscessed incisor with an incompletely formed apex. The central incisor of a boy, age six and one-half, was fractured and the pulp became abscessed. The tooth was treated with a dressing of CMCP and sealed with zinc phosphate cement. The tooth was treated until three negative cultures were obtained. The root canal was then filled

with a powder composed of zinc oxide, thymol iodide, white rosin and paraformaldehyde mixed with glycerite of iodine liquid. The filling material was forced apically until a pain response was elicited from the patient indicating filling to vital tissue. Easlick showed successive radiographs which demonstrated continued formation of the root end. Seven months after treatment the apex of the tooth had completed its formation.

Herbert,[81] reporting in 1959, showed radiographic evidence of periapical repair and occlusion of the apex of a tooth which had a divergent apex and a chronic fistula draining from a periapical radiolucent area. The tooth was treated with a polyantibiotic paste placed in the apical root canal. The coronal portion of the canal was filled with a mixture of zinc oxide and oil of cloves. A radiograph, five years later, showed repair of the periapical lesion and a normal periodontal membrane. The root canal appeared completely closed by formation of an apical radiopaque mass. The tooth remained symptomless. He presented another report of a tooth with arrested development and periapical infection treated in the same manner. A four year follow-up radiograph demonstrated repair of the periapical area with bone. However, no further root formation was observed in this tooth.

In 1960, Cooke and Rowbotham[10] reported a technique for treatment of pulpless teeth with open apices. Their clinical study covered a period of 10 years. The technique advocated by the authors is cleansing of the canal in the routine manner to remove

necrotic debris after any acute symptoms have subsided. A dressing of tricresol/formalin or beechwood creosote is then sealed in the canal. The treatment is repeated at weekly intervals until no exudate is present or it has been reduced to a minimum and the tooth is free of symptoms. At this time an antiseptic paste is inserted into the root canal and carried to within several millimeters of the apical foramen. The root canal is then sealed with cement and an amalgam filling. The antiseptic paste consists of the following materials:

zinc oxide	64 parts
cresol	16 parts
oil of caryoph	16 parts
iodoform	3.5 parts
thymol	0.5 parts

The material is mixed to a soft buttery consistency. The authors stress the point that none of the material should pass into the periapical tissues. The patient is recalled at six month intervals to examine the tooth radiographically. According to Cooke and Rowbotham, this technique will allow many teeth with incomplete development to be retained in a healthy condition without having to resort to surgical procedures. When the patient is older and the cementum is less permeable an apical resection may be performed. However, in many of these teeth, there will be continued apical development following treatment. When apical development has occurred, the antiseptic paste is removed from the canal and a conventional root filling is inserted. The authors speculated that in those teeth in which continued apical development occurred

that the epithelial sheath of Hertwig was not irreparable damaged. When infection was removed, the sheath continued its function of root formation. Since the study contained no histologic evidence, the role of Hertwig's epithelial sheath could not be proven.

In 1961, Nygaard Ostby[82] presented radiographic evidence of continued apical development in pulpless teeth after stimulating bleeding into the canal by lacerating granulation tissue outside the foramen. After stimulating hemorrhage into the canal, the cervical portion of the canal was sealed with a short gutta-percha point coated with Kloroperka paste. In teeth with completed apices treated in a like manner, Ostby demonstrated the ingrowth of granulation tissue from the periapical areas into the canals. However, this organization did not proceed far into the canal, even when not limited by the root filling. The granulation tissue was gradually transformed into fibrous connective tissue. The fibrous tissue formed could not be distinguished from that found after a partial pulp extirpation.

Matsumiya and his coworkers,[56] reporting in 1962, demonstrated histologic evidence of the formation of a "cementum-or bone-like tissue" subsequent to the filling of root canals with a "calcium hydroxide paste." Sections are shown demonstrating the formation in dog's and human teeth. This phenomenon occurred whether the root canal was infected or uninfected. According to the authors, the phenomenon is a result of the self-defense function of the periapical tissues against harmful stimulations that invade the

tissues from the root canal. They divided this sealing of the root canal into three groups, those closing inside the root canal, those closing at the apical foramen and those closing outside of the root apex. The authors stated that the use of "calcium hydroxide paste" actively induced the proliferation of granulation tissue and its cicatrization. The formation of "cementum-or bone-like structure" and the regeneration of alveolar bone are remarkably accelerated.

In 1963, Moodnik[83] reported a technique of treating teeth with open apices which exhibited periapical breakdown. In his technique, Moodnik advocates removal of the greatest bulk of tissue irritants from the canal. Granulation tissue which is near the apical foramen is left undisturbed. As granulation tissue is a necessary precursor to healing, removal of this tissue is neither necessary nor desirable. The coronal portion of the root canal is then obturated. In a series of some 50 teeth which were filled in this manner, Moodnik reported 80 per cent success. The remaining 20 per cent were treated after failure of the technique by apical resection.

Ball,[11] reporting in 1964, demonstrated radiographic evidence of continued apical formation after the treatment of a permanent central incisor with an acute abscess of the pulp. After the acute phase subsided, he cleansed the canal and placed a radiopaque antibiotic paste in the root canal to within two millimeters of the apex. The tooth was then sealed with a zinc oxide - eugenol

paste. Radiographically, he showed a sealing of the root apex with the deposition of a calcified material about the apex.

In 1965, Crabb[84] reported a case in which he radiographically demonstrated apical closure of a tooth with a radiopaque mass and the reduction of a periapical radiolucency. The permanent incisor presented with an area of radiolucency surrounding the incompletely formed apex. The tooth was treated by cleansing the canal with reamers, followed by disinfecting with CMCP. The root canal was filled with a paste composed of calcium hydroxide and distilled water. Deposition of a radiopaque mass appeared to have obliterated the apical foramen and the periapical radiolucency was no longer visible after one year.

In 1964, Kaiser[85] presented, for the first time, the use of a paste composed of calcium hydroxide and CMCP in the treatment of pulpless permanent teeth with divergent apices. Since 1958, Kaiser[86] has treated over 50 teeth by placement of the paste in the canal after the canal is rendered sterile. The canal is disinfected by the use of CMCP. Sterility is assessed by a culture. The patient is recalled every six months and observed radiograhically. When radiographic evidence of apical closure is observed, the paste is removed and a permanent filling of gutta-percha is placed. By surgically removing the apical portion of treated teeth and making histologic sections, Kaiser has demonstrated the deposition of a cementoid type material closing the apical foramen. Minute openings communicating with the periapical tissues persist

in some sections. It is because of the possibility of these minute communications with the apical tissues that Kaiser routinely fills the teeth with gutta-percha after radiographic evidence of closure of the apical foramina.

In 1965, Natkin[87] described the technique used by Frank[9] to induce apical closure in pulpless teeth with divergent apices. Natkin presented four maxillary incisors treated by Frank to demonstrate the apical closure which had occurred after placement of a paste of calcium hydroxide mixed with CMCP.

Frank,[9] reporting in 1966, described his technique for treatment of the pulpless tooth with an underdeveloped root having a divergent apex. The gross necrotic material is removed from the canal by filing and frequent irrigation with sodium hypochorite. The canal is dried and a thick paste of calcium hydroxide and CMCP is packed into the canal. The tooth is then sealed and followed radiographically every three to six months. If symptoms develop the material is removed and the procedure is repeated. When evidence of apical closure is apparent on the radiograph, the closure is verified by opening into the canal and testing with an instrument. When the apex is closed, or a better designed apex which will permit routine filling is formed, the dressing is removed and a complete filling of gutta-percha with lateral condensation is placed. Frank reported four different types of apical closure which he had observed clinically. The first of these is a closure of the apex with a slight recession of the root canal. The second

form is obliteration of the apex without any change in the root canal space. The third type of apical closure shows no radiographic evidence of obliteration. However, a thin calcified bridge has developed across the apex which will allow for the filling procedure. The final type of apical obliteration is a calcified bridge which forms just coronal to the apex and can be seen on a radiograph. Frank presented radiographs of six teeth treated successfully with his technique.

In 1966, Bouchon[88] published a report showing continued apical formation in a permanent incisor with a necrotic pulp following the insertion of Walkoff's paste. The root canal was sterilized with cresanol before placement of the paste. A radiograph taken five months after treatment showed continuing apical development. Fifteen months after treatment, a radiograph showed a fully formed apex. The canal was then filled with gutta-percha. A radiograph taken one year later demonstrated the periapical area completely healed and a healthy periodontal membrane surrounding the tooth.

Feldman, Solomon and Notaro,[89] reporting in 1966, advocated zinc oxide-eugenol as the filling material in treatment of "blunderbuss" apices of teeth with necrotic pulps. The canal is cleansed in the usual manner. Sterile zinc oxide-eugenol is placed in the canal and teased to the apex. Small pieces of gutta-percha are pushed apically to serve as a plugger for the zinc oxide-eugenol cement. The filling is carried just short of the apex in order to allow the best chance for biologic closure. Radiographs are

presented which show successful treatment of a tooth with this technique. The authors state that it is reasonable to expect a greater incidence of failure when treating pulpless teeth with incompletely formed roots. When the conservative technique recommended by the authors, fails, they recommend a surgical approach to seal the apex of the tooth.

In 1966, Friend[90] reported a study in which root canal therapy was performed on 87 teeth with open apices in patients aged between seven and 28 years. The teeth were selected when pulpotomy procedures were unsuccessful or contraindicated. The teeth were judged to have an open apex when a number 12 reamer was loose at the apex when first inserted into the canal. Of the 87 teeth treated, 11 had divergent apices, 20 had parallel dentinal walls and 56 had tapering dentinal walls. The root canals were cleansed and chloramphenicol paste was sealed in the canal to render the canal sterile. When the clinical condition of the tooth and the bacteriological culture were satisfactory, the root canal was filled with Diatek (a compound of betadiketones with zinc oxide). The material was mixed to a creamy consistency and a large rotary paste filler was used to carry the material to the apex. A permanent filling was then placed. The teeth were then examined at three months, nine months and then at yearly intervals. At follow-up periods ranging from six months to three and one-half years, 90 per cent of the teeth remained satisfactory when judged from a clinical and radiographic basis. In 20 of the teeth radio-

graphic evidence of continued apical growth was found or calcification had taken place across the end of the root filling material.

Michanowicz and Michanowicz,[91] reporting in 1967, described a technique for filling the root canal of teeth with divergent apices which had become pulpless. In their techniques, the walls of the coronal and middle thirds of the root canal are ground with a sterile diamond stone to make the walls as parallel as possible. The root canal is cleansed and a dressing of CMCP is sealed in the canal. When two negative cultures are obtained, the canal is filled. A primary gutta-percha point is fabricated. Calcium hydroxide and sterile water are mixed to a creamy consistency and carried to the apex on a number 12 sterile plugger. Root canal sealer is then carried to the canal on a lentulo spiral instrument and the walls of the canal are coated with the sealer. The apical end of the primary cone is coated with a thick mix of the canal sealer and carried to place. The canal is filled by means of lateral condensation. Very little pressure is exerted in an apical direction in an effort to limit the extrustion of the filling material beyond the apex. Radiographs are shown demonstrating a tooth treated with their technique. Apical root formation encompassing the root canal filling material at the apex of the tooth was demonstrated in a radiograph taken two years after treatment.

Day,[62] in 1967, published a case report of continued apical root formation in a pulpless tooth following the insertion of a calcium hydroxide root canal filling. The maxillary central incisor of a girl, age 10, presented with tenderness, looseness and radiographic evidence of a large area of periapical bone destruction. The tooth was cleansed and a polyantibiotic paste consisting of penicillin, streptomycin and sodium caprylate was placed in the canal. After three months the culture was negative and a suggestion of bony repair was evident radiographically. The tooth was then filled with a calcium hydroxide paste. Nine months later, radiographic evaluation showed a calcified barrier closing the apex. The canal was cleaned again and a gutta-percha filling was placed. The tooth was later moved orthodontically and remained healthy. Radiographic follow-up four years after treatment revealed further development of the root. The anatomy of the surrounding tissue had returned to normal.

Frank,[93] reporting in 1967, again described his technique of filling pulpless teeth with underdeveloped roots. He presented additional cases to demonstrate apical closure in pulpless permanent teeth after filling the canal with a paste composed of calcium hydroxide mixed with CMCP. In reviewing other techniques used to stimulate apical closure, Frank concluded that the effectiveness of the medication used is relatively unimportant. The important factors are cleansing of the canal by instrumentation and medication and a reduction of the canal space with a

temporary filling material. These changes allow an improvement in the apical environment which will permit continuation of apical development.

In 1968, Steiner, Dow and Cathey,[94] reported four case histories showing radiographic root end closure of pulpless teeth following treatment with calcium hydroxide and CMCP. The technique used in treatment of the teeth was identical to that of Frank[9] except a dressing of CMCP was sealed into the teeth after cleansing and the canals were filled with the paste on the next appointment. The response reported by the authors was identical to that reported by Frank.[9, 93]

STATEMENT OF PROBLEM

This investigation was undertaken to study the histologic responses in the periapical areas of pulpless teeth with incompletely formed roots and divergent apices when treated with two different root canal filling pastes. These pastes consisted of: (1) calcium hydroxide mixed with camphorated parachlorophenol, and (2) calcium hydroxide mixed with distilled water.

Erupted developing permanent teeth of young mongrel dogs were used in the experiment. A vital dye was injected in order to demonstrate the formation of calcified tissues after treatment. Semi-serial histologic sections through the teeth and periapical tissues were studied.

EXPERIMENTAL PROCEDURE

A litter of six mongrel dogs, of known birth date, with erupted deciduous teeth were selected for this study. The development of the crowns and roots of the permanent teeth in these animals were followed from radiographs made beginning at age three months. The periodic radiographs revealed loss of the deciduous incisors between three to four months of age. The deciduous molars were lost between ages five to six months. The permanent incisors and the first premolars erupted into the oral cavity during the fourth month. The remaining premolars erupted into the oral cavity during the fifth month after birth. At the time treatment of the teeth was initiated, the dogs lacked five days of being six months old.

After evaluation of the radiographs the following 10 permanent teeth in each of the six dogs were chosen for control observations and treatment (Figures 1 and 2). These teeth were the maxillary second and third premolars, and the mandibular second, third and fourth premolars. This provided a total of 60 young permanent teeth with the same level of almost complete root apex closure. The erupted permanent teeth used in this experiment were selected when they demonstrated the following criteria: radiographic evidence of (1) incompletely formed roots having divergent apices, (2) appropriately arranged root and bone position to provide well oriented histologic sections, and (3) accessibility of the tooth in the mouth to facilitate treatment.

The five maxillary and mandibular teeth on one side of the mouth of one dog were left untreated to observe the normal development of the apical regions of the teeth. The pulps of the remaining selected group of 55 teeth in this and the other five dogs were exposed with a #6 round carbide bur and an air driven high speed handpiece. The entire roofs of the pulp chambers were removed with the bur. All operations were conducted with the dogs under general anesthesia. Anesthesia was induced by intravenous Nembutal Sodium* with a dosage of one milliliter for each five pounds of body weight.

The approximate lengths of the root canals of all of the teeth were established from the preoperative radiographs. Root canal files were marked at these lengths and inserted into the root canals of each of the 55 teeth used for treatment and control. Radiographs were taken of the teeth with the files in place within the root canals. Using the radiographs with the files inserted to known lengths, calculations of the correct lengths of the root canals of the exposed control and treatment teeth were made by the formula:

$$\frac{\text{x-ray length of root canal}}{\text{x-ray length of file}} = \frac{\text{actual length of root canal}}{\text{actual length of file.}}$$

The correct lengths of each of the canals were recorded.

The pulps of the experimental teeth were further lacerated with a barbed broach and contaminated with the dogs' saliva. All of the exposed pulp canals of the 55 experimental teeth were left open to the oral environment for one week.

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*Abbott Laboratories, Chicago, Illinois.

The dogs were injected with an aqueous solution of Procion brilliant red H-8BS* intraperitoneally two days after the tooth pulps had been exposed and the root canals measured. The vital dye was injected in order to demonstrate formation of any tooth and periapical calcified tissue that would be added after treatment of the young teeth. The Procion dyes were originally described as in vivo hard tissue marking agents by Goland and associates.[95] Tomich[96] has shown that a dosage of 100 milligrams of Procion per kilogram of body weight is an effective hard tissue marker and that dosage was used in this study.

At the end of one week after the pulp canals had been measured and exposed to oral infection, one dog was sacrificed to show the extent of the necrosis and inflammation. These teeth were used to assess the existing conditions of the experimental teeth and their surrounding periapical tissues at the time of treatment. Since all the dogs involved in the study were siblings and the radiographic surveys revealed similar dental findings, it was felt that the apical histology of this animal would be the same as that of the other experimental animals.

The jaws of the sacrified animal were removed and placed temporarily in 10 per cent formalin. Individual teeth and immediate periapical tissues were separated by cutting through the jaw with a band saw in a facial to lingual plane. Each individual tooth and its surrounding tissue was then replaced in 10 per cent formalin to complete fixation.

*I. C. I. Organics Inc., Providence, Rhode Island.

The teeth of the experimental dogs, the root apices of which were to be filled with one or the other of the therapeutic calcium hydroxide pastes, were isolated with a rubber dam. The root canals of all of the teeth to be filled were mechanically cleaned with root canal files. The canals were frequently irrigated with Zonite* (sodium hypochlorite) and three per cent hydrogen peroxide.† The canals were cleansed of all gross necrotic material. Paper points were used to dry the canals. All the selected teeth were completely treated in this manner except five teeth mentioned above in one dog's mouth. These five teeth received no treatment except exposure and laceration of the pulp. These teeth were left untreated to observe changes in the periapical region.

The teeth were randomly selected to receive one of two root canal filling pastes: (1) calcium hydroxide powder‡ mixed with camphorated parachlorophenol§ or (2) calcium hydroxide powder mixed with distilled water. The pastes were mixed to a putty-like consistency on a glass slab and placed in the teeth with an amalgam carrier. A large root canal spreader with the end blunted and marked according to the length of the canal was used to force the material to the apex. An effort was made to completely fill the canal with the paste without forcing an excess through the

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* Chemway Corporation, Dunbar Laboratories, Wayne, New Jersey.
† Kent Drug Co., Allegon, Michigan.
‡ J. T. Baker Chemical Company, Phillipsburg, New Jersey.
§ J. Bird Moyer Co. Inc., Philadelphia, Pennsylvania.

apical foramen. When the canals were completely filled, the coronal portions of the canals were cleaned with a spoon excavator. A double seal of Cavit* and zinc phosphate cement was used to seal the openings into the root canals.

The monthly radiographic surveys were continued after treatment. The radiographs were studied for any evidence of apical closure (Figures 1a and 2a).

The remaining five animals were sacrificed after a four month observation period. After fixation, the teeth and surrounding tissues were decalcified in 10 per cent formic acid for two to three weeks, and dehydrated in 30 to 100 per cent alcohol for 12 to 24 hours at each concentration. Naphtha was used to remove residual alcohol from the tissues. The tissues were placed in melted paraffin for 48 hours, and paraffin blocks containing the tissues were prepared. Seven micron semi-serial sections were prepared and routinely stained with hematoxylin and eosin. Alternate sections were left unstained for viewing with fluorescent light microscopy.

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*Premier Dental Products Company, Philadelphia, Pennsylvania.

DATA

Histologic Evaluation of Uninfected Teeth

Five teeth were left untreated throughout the length of the study. These teeth and the surrounding periapical tissues were examined microscopically to determine the normal histologic features of the apex of a completely formed dog's tooth (Figure 3). The pulpal walls of the root canals were slightly convergent apically from the cervical area to the apex. Except for multiple minute apical foramina communicating with the periapical tissues, the apex of each of the root canals was closed by cellular cementum (Figure 3a). The cementum was, on the average, approximately 25 microns thick at the junction of the middle and apical portions of the root. A gradual increase in the thickness of the cementum from this area of the root was observed with an average thickness of approximately 350 microns at the apex of the tooth.

Observation of the unstained sections with fluorescent light microscopy revealed marking of the dentin and cementum by the Procion dye. Fluorescent markings demonstrated that at the time of injection of the dye, the roots of the teeth were incompletely developed and had divergent apices (Figure 3b). The markings also indicated that following the injection dentin and cementum formation closed the apices of the roots except for multiple minute apical foramina communicating with the periapical tissues.

Histologic Evaluation of Infected Teeth

The pulps of 10 control teeth had been exposed and left open to the oral environment for one week. These teeth were examined microscopically to evaluate the root development and the state of the pulp and periapical tissues. With the exception of one tooth (Figure 4) the full length of the roots had been established and Hertwig's epithelial root sheath was no longer apparent. All roots were incompletely developed and had divergent apices with wide-open apical foramina (Figure 5). A slight deposition of cementum was noted laterally on the apical one-third of each root.

Under the microscope, all pulps were partially necrotic but all had vital apical tissue present. In four of the 10 teeth abscesses extended apically one-fourth to one-third the lengths of the root canals. Dense collections of polymorphonuclear leukocytes with areas of focal necrosis were present. The approximating pulp showed histological evidence of inflammation and small areas of extravasated blood. An appreciable portion of pulp tissue in the apex of each of the four teeth appeared relatively normal.

The root canals of the other six teeth contained abscesses extending one-half to three-fourths the lengths of the root canals (Figure 5). Dense collections of polymorphonuclear leukocytes with areas of focal necrosis were present. The inflammatory reaction extended into the periapical tissues. The apical pulp and immediate periapical tissues showed a diffuse leukocytic infiltra-

tion of plasma cells, lymphocytes and occasional polymorphonuclear leukocytes (Figures 5a and 5c). The inflammation at the apices was classified as low grade, chronic. Blood vessels appeared engorged and contained many leukocytes. Small areas of extravasated blood were present in the pulp and periapical tissues.

Observation of unstained sections with fluorescent light microscopy revealed a marking of the tissues undergoing calcification at the time of the Procion dye injection. A perfect correlation existed between the dentin and cementum with dye markings in the unstained sections and that observed to be undergoing calcification in the hematoxylin and eosin stained sections (Figures 5a and 5b).

Histologic Evaluation of Treated Teeth

The connective tissues around the roots of the treated teeth were examined microscopically and the following two conditions were evaluated: (1) formation of calcified material at the apex of the tooth, and (2) the inflammatory response of the apical tissues.

The formation of calcified tissues at the apices of the teeth was classified as follows:

1. Complete-Complete closure of the apical foramen by the formation of calcified material except for microscopic communications with the apical tissues.
2. Incomplete-Formation of calcified material over one-third to three-fourths of the apical foramen.

3. Slight-Formation of any calcified material evident but less than one-third of the apical foramen closed by the formation.

4. None-No formation evident.

The inflammatory response of the apical tissues was classified according to the following criteria:

1. None-No inflammation present

2. Mild-Small granuloma with a few scattered plasma cells and lymphocytes present.

3. Moderate-Granuloma present with predominance of inflammatory cells being polymorphonuclear leukocytes.

4. Severe-Areas of focal necrosis with the predominant cell being polymorphonuclear leukocytes.

Filling Material: Calcium Hydroxide and Camphorated Parachlorophenol.

Microscopic examination of 42 roots of teeth treated with calcium hydroxide - CMCP revealed all four classifications of apical formation of calcified tissue. All four classes of inflammation were observed (Table I). Table III associates the frequency of occurrence of apical formation and inflammation in these roots.

Class 1

Complete closure of the apical foramina by the formation of calcified tissue resembling cementum occurred in 10 of the 42 treated roots (Figures 6 and 6a). Two other roots demonstrated a complete closure except for several microscopic communications with the apical tissues (Figure 7b). The calcified tissue was continuous with the cementum deposited laterally on the root of each tooth (Figures 6 and 7). The calcified tissue was surrounded by fibrous connective tissue resembling normal periodontal ligament. The apical tissues were free of inflammation (Figure 7c).

The unstained sections were evaluated with fluorescent light microscopy. The fluorescent markings of the Procion dye were evident in the roots of the teeth. However, no marking was present in the calcified tissue closing the apices (Figure 7b). Therefore, it was concluded that the calcified deposition was formed after treatment of the teeth.

Class 2

Four roots demonstrated an incomplete closure of the apical foramina by formation of a cementum type material over the apices. In three specimens, blood clot, composed of fibrin and red blood cells, appeared to interfere with complete closure. Foreign body giant cells were demonstrated surrounding the clot. The apical tissues were free of inflammation in three specimens. The apices of these roots were surrounded by fibrous connective tissue resembling normal periodontal ligament. An inflammatory reaction of the tissue surrounding the apex of the fourth root was classified as mild. A small granuloma with a few scattered plasma cells, lymphocytes and an occasional polymorphonuclear leukocyte was observed at the apex of this root. A thin peripheral band of normal connective tissue was present between the alveolar bone and the granulation tissue.

Cellular cementum with a thickness equivalent to that found in the untreated tooth was present laterally on the apical one-third of each of the four roots. Evaluation of the unstained sections with fluorescent microscopy showed that this cementum was formed after the teeth were treated.

Class 3

Seven roots demonstrated a slight closure of the apical foramina by the deposition of a slight amount of calcified tissue on the inner walls of the apical foramina. The tissue resembled cementum. In five specimens, blood clot appeared to interfere

with deposition of calcified tissue. Foreign body giant cells were observed surrounding the clot on the apical aspect of the material in these five specimens.

Of the seven specimens, the connective tissues surrounding the apices of five were free of inflammation. The tissues resembled normal periodontal ligament. The apices of two of these roots were surrounded by small granulomas. A few scattered plasma cells, lymphocytes and an occasional polymorphonuclear leukocyte were noted within the granulation tissue. The inflammatory reaction was judged to be mild.

The deposition of cementum laterally on the roots was equivalent to that present on the untreated teeth. With the aid of the Procion dye in unstained sections viewed under fluorescent microscopy, it was shown that the cementum had been deposited after treatment of the teeth.

Class 4

Nineteen of the roots treated with calcium hydroxide and CMCP showed no formation of calcified tissue at the apices of the roots. Of these, two demonstrated no inflammatory reaction within the apical tissues. The presence of blood clot was noted directly within the wide-open apical foramina (Figure 8). A foreign body reaction with foreign body giant cells was observed in the connective tissue beneath the clot (Figures 8a and 8b). The apices of the two roots were surrounded by fibrous connective tissue

resembling normal periodontal ligament. The active deposition of alveolar bone was noted. Osteoblasts lined the alveolar bone surrounding the apex (Figure 8b).

Six of the roots of teeth of this group demonstrated a mild apical inflammatory reaction. These apices were surrounded by small granulomas which extended slightly into the wide-open apical foramina. In two of these roots, the granulation tissue extended approximately one-third of the way up the root canal. Numerous foam cells were present in the granulation tissue. A few scattered plasma cells, lymphocytes and polymorphonuclear leukocytes appeared within the granulomas. A slight amount of resorption of apical tooth structure was visible. The apical foramen of one of these roots was filled with blood clot. Numerous foreign body giant cells were noted in the connective tissue beneath the clot.

The connective tissue response of six roots of this group was judged as moderate. Granulomas with numerous polymorphonuclear leukocytes were present. The granulomas surrounded the apices of the roots and in all but one specimen extended through the wide-open apex two to three millimeters into the root canal (Figure 9). In one specimen the granulation tissue extended approximately one-third the length of the root canal. Numerous foam cells containing dark-staining particles were present within the granulation tissue. In two root canals a small area between the filling material and the granulomas was occupied by blood clot.

A foreign body giant cell reaction was present in the granulation tissue adjacent to the clot. The apices of all six roots had undergone a slight amount of resorption of dentin and cementum. Active resorption of tooth root and bone was noted.

The apical inflammatory reaction of five roots was judged to be severe. Granulomas measuring two to three millimeters in diameter were present surrounding the apices. Many polymorphonuclear leukocytes and areas of focal necrosis were present within the granulation tissue. In all specimens the granulation tissue extended through the wide-open apical foramina two to three millimeters into the canal. Debris was present in the root canals between the granulomas and the filling material. The debris included pulp tissue which was not removed during the cleansing procedures. Polymorphonuclear leukocytes and red blood cells mixed with filling material were present in large numbers within the root canals. The granulation tissue near the debris was highly vascular.

An island of filling material mixed with blood clot was noted within one of the granulomas. The material had obviously been forced through the apical foramen of the tooth during the filling procedure. The material was surrounded by many multinucleated giant cells.

Resorption of dentin and cementum was noted at the apices of these five roots. The granulomas were expanding by active alveolar bone resorption. Numerous osteoclasts were present in areas

of resorption.

Deposition of cellular cementum on the lateral surfaces of the 19 roots of teeth in this group was observed to be roughly the same regardless of the inflammatory reaction. The fluorescent markings of the Procion dye revealed that this cementum was formed after treatment of the teeth.

Filling Material: Calcium Hydroxide and Distilled Water.

Microscopic examination of 19 roots of teeth treated with calcium hydroxide and distilled water revealed all four classifications of apical formation of calcified tissue. Likewise, all four classes of inflammation were observed (Table II). Table IV associates the frequency of occurence of apical formation and inflammation in these roots.

Class 1

Complete closure of the apical foramina by the formation of a calcified tissue resembling cementum occurred in two of the 19 roots treated in this manner (Figure 10). In these two specimens, the calcified tissue was continuous with the cementum deposited laterally on the root surfaces. The apical tissues were free of inflammation. The apices of the two roots were surrounded by fibrous connective tissue resembling normal periodontal ligament (Figures 10a and 10c).

Evaluation of the unstained sections with fluorescent light microscopy showed no Procion marking in the calcified tissue closing the apices of the two teeth (Figure 10b). The fluorescent marking demonstrated that the apical foramina of the teeth were divergent when treated and apical closure had developed subsequent to the treatment of the teeth.

Class 2

Only one of the 19 roots treated with calcium hydroxide and distilled water demonstrated an incomplete closure of the apical

foramen by the deposition of a cementum type tissue over the apex. This calcified tissue was continuous with the cementum on the lateral surface of the root, but failed to close the central third of the apical foramen. The apex of the root was surrounded by fibrous connective tissue resembling normal periodontal ligament. The apical tissues were free of inflammation.

Class 3

Five roots demonstrated a slight attempted closure of the apical foramina by the deposition of a slight amount of calcified tissue at the apices. The calcified tissue resembled cementum. The connective tissue surrounding one root appeared normal and was free of inflammation.

On the other hand, four specimens showing only slight or attempted root closure also showed an inflammatory reaction judged as mild. In this group, filling material extended through the wide-open apical foramen due to an over-filling of the canals in three of the teeth (Figures 11 and 11a). Very small granulomas were present apical to the filling material. A few scattered plasma cells and lymphocytes could be identified within the granulation tissue. Numerous macrophages were present.

In two specimens, in the areas where calcified tissue had not been formed, blood clot was present between the filling material and the connective tissue. Many multinucleated giant cells surrounded the blood clot.

Examination of the unstained sections under the fluorescent microscope showed no Procion markings in the calcified tissue. Therefore, it was concluded that the calcified tissue in the periapical tissues was formed as a result of the treatment. The deposition of cementum on the lateral surfaces of the roots after treatment was roughly equivalent to that present on the untreated teeth.

Class 4

In 11 of the 19 roots treated with calcium hydroxide and distilled water there was no evidence of closure of the apices by calcification of the periapical tissue. However, active cementum deposition laterally on the existing root surfaces was noted for all of the teeth in this group (Figure 12). The amount of cementum formed was equivalent to that formed laterally on the existing root apices of the untreated teeth. The Procion dye marking in the unstained sections viewed with fluorescent microscopy demonstrated that this cementum had been formed after treatment of the teeth (Figures 12a and 13b).

Inflammatory reactions ranging from mild to severe were present in the periapical tissues. The inflammatory reaction around four of the roots was considered as mild (Figure 12). Blood clot was present between the filling material and the granulation tissue in many areas. Foreign body reactions with numerous multinucleated giant cells were present around the areas of clot.

The granulation tissue contained many macrophages. A few scattered plasma cells, lymphocytes and polymorphonuclear leukocytes were noted in the periapical tissues. Areas of active resorption of alveolar bone, dentin, and cementum were occurring adjacent to the granulomas.

The periapical tissues of three of the roots had a moderate reaction to the filling material. Granulomas surrounded the apices of the three roots. In two of the specimens the periapical granulation tissue extended through the apical foramina and into the apical one-thirds of the root canals. Numerous polymorphonuclear leukocytes were present. The granulomas were expanding about the apices by the active resorption of alveolar bone, cementum and apical dentin. Numerous osteoclasts were observed. An area of blood clot was noted filling the apical foramen of the third root. The clot occupied the space between the filling material and the granulation tissue. A foreign body reaction with multinucleated giant cells was present in the granulation tissue adjacent to the clot. Polymorphonuclear leukocytes were numerous within the granuloma. Areas of active resorption of alveolar bone, apical dentin and cementum were present.

The inflammatory reaction was severe surrounding four roots filled with calcium hydroxide and distilled water. Large areas of bone resorption were present surrounding the apices of the roots (Figure 13). Granulomas occupied the areas of bone resorption and extended into the root canals one-third to one-half the

lengths of the roots. Necrotic debris was present within the root canals coronal to the granulation tissue. The debris was composed of necrotic pulp tissue, hemorrhage and pus.

Active resorption of alveolar bone, dentin, and cementum was noted. Many osteoclasts were noted in Howship's lacunae along resorbing bone, dentin and cementum margins.

The predominant cell within the granulomas was the polymorphonuclear leukocyte (Figure 13a). Numerous areas of focal necrosis containing pus were evident within the granulation tissue. There were numerous capillaries and many vacuolated foam cells containing granules.

Evaluation of Teeth with Pulp Exposure Left Open During Study Period.

Clinical Evaluation

The pulps of five teeth were exposed to the oral fluids during the study period. These teeth received no treatment after exposure of the pulps. One of the teeth was exfoliated between the third and fourth month of observation. The remaining four teeth were extremely mobile and had no bony support. Radiographs showed a destruction of the alveolar bone surrounding the teeth.

Histologic Evaluation

The remaining six teeth had no bony support and were attached to bone only by granulation tissue (Figure 14). Areas of gross resorption of alveolar bone and tooth root were noted. Areas of focal necrosis containing pus surrounded the remaining surfaces of

the roots. Patent communications from the apical areas to the oral cavity were demonstrated. Dense collections of polymorphonuclear leukocytes were present in the surrounding granulation tissue. It was apparent from the histologic picture that the teeth were near exfoliation.

Statistical Analysis

The chi-square test was used to detect the association between apical formation and inflammation. The chi-square test was found to be significant and showed an association between the two variables. The coefficient of contingency was then computed. The coefficient of contingency[97] is a measure of the degree of correlation for categorical data between two related variables.

Because of the absence of cases in some cells in the original tables (Table III and IV), some of the categories were combined for chi-square tests to be valid. The original Tables III and IV were reduced to form a two by three contingency table for these teeth treated with calcium hydroxide and CMCP, and a two by two contingency table for those teeth treated with calcium hydroxide and distilled water, as shown in Tables V and VI.

For the calcium hydroxide and CMCP treated group, the observed chi-square with two degrees of freedom was highly significant ($P < .001$). The coefficient of contingency (C) was calculated to be 0.62 (see Table V) showing a significant relationship between apical closure and inflammation.

For the calcium hydroxide and distilled water treated group, the observed chi-square with one degree of freedom was significant ($P < .005$). The coefficient of contingency was calculated to be 0.55 (see Table VI) showing a significant relationship between the two variables studied.

TABLES AND ILLUSTRATIONS

TABLE I

Teeth Treated with Calcium Hydroxide and Camphorated Parachlorophenol. Observation Period Four Months.

Section	Apical Closure	Inflammation			
		None	Mild	Moderate	Severe
1171-a	-				X
1171-b	-				X
1174	++	X			
1177	-			X	
1215-a	+++	X			
1215-b	+++	X			
1216	-		X		
1219	-		X		
1224	+		X		
1226	+++	X			
1227	+++	X			
1228-a	+	X			
1228-b	-		X		
8454-a	+++	X			
8454-b	-			X	
8455-a	-			X	
8455-b	-			X	
8457-a	-			X	
8457-b	-		X		
8459-a	-				X
8459-b	-				X
8460-a	-	X			
8460-b	+	X			
8461-a	++	X			
8461-b	+++	X			
8473-a	-	X			
8473-b	++	X			
8474-a	+++	X			
8474-b	+	X			
8480-a	-		X		
8480-b	+	X			
8484	+		X		
8487-a	++		X		
8487-b	+++	X			
8493-a	-				X
8493-b	-		X		
8495-a	-			X	
8495-b	+	X			
8496-a	+++	X			
8496-b	+++	X			
8497-a	+++	X			
8497-b	+++	X			

\- No Apical Closure
\+ Slight Apical Closure
++ Incomplete Apical Closure
+++ Complete Apical Closure

TABLE II

Teeth Treated with Calcium Hydroxide and Distilled Water.

Observation Period Four Months.

Section	Apical Closure	Inflammation			
		None	Mild	Moderate	Severe
1176	++	X			
1229	-			X	
1230	-			X	
8453-a	-				X
8453-b	-				X
8458-a	-				X
8458-b	+		X		
8475-a	-		X		
8475-b	-		X		
8477-a	+	X			
8477-b	-				X
8482-a	+		X		
8482-b	+	X			
8483-a	+		X		
8483-b	-		X		
8488-a	+++	X			
8488-b	-		X		
8494-a	-		X		
8494-b	+++	X			

\- No Apical Closure
\+ Slight Apical Closure
++ Incomplete Apical Closure
+++ Complete Apical Closure

TABLE III

Association of Inflammation and Apical Closure in Teeth Treated with Calcium Hydroxide and CMCP.

Apical Closure	Inflammation				Total
	None	Mild	Moderate	Severe	
None	2	6	6	5	19
Slight	5	2	0	0	7
Incomplete	3	1	0	0	4
Complete	12	0	0	0	12
Total	22	9	6	5	42

TABLE IV

Association of Inflammation and Apical Closure in Teeth Treated with Calcium Hydroxide and Distilled Water.

Apical Closure	Inflammation				Total
	None	Mild	Moderate	Severe	
None	0	4	3	4	11
Slight	1	4	0	0	5
Incomplete	1	0	0	0	1
Complete	2	0	0	0	2
Total	4	8	3	4	19

TABLE V

Two by Three Contingency Table for Teeth Treated with Calcium Hydroxide and CMCP.

Apical Closure	Inflammation			Total
	None	Mild	Moderate and Severe	
None	2	6	11	19
Slight, Incomplete and Complete	20	3	0	23
Total	22	9	11	42

$\chi^2 = 26.59$ ($P < .001$)
$C = 0.62$

TABLE VI

Two by Two Contingency Table for Teeth Treated with Calcium Hydroxide and Distilled Water.

Apical Closure	Inflammation		Total
	None and Mild	Moderate and Severe	
None	4	7	11
Slight, Incomplete and Complete	8	0	8
Total	12	7	19

$\chi^2 = 8.06$ ($P < .005$)
$C = 0.55$

Figure 1a: Preoperative maxillary lateral jaw radiograph of dog. Note the wide-open apical foramina.

Figure 1b: Four months postoperative radiographs of same area as Figure 1a. The four canals of the second and third premolars were filled with a paste of calcium hydroxide and CMCP.

A. Apparent apical closure

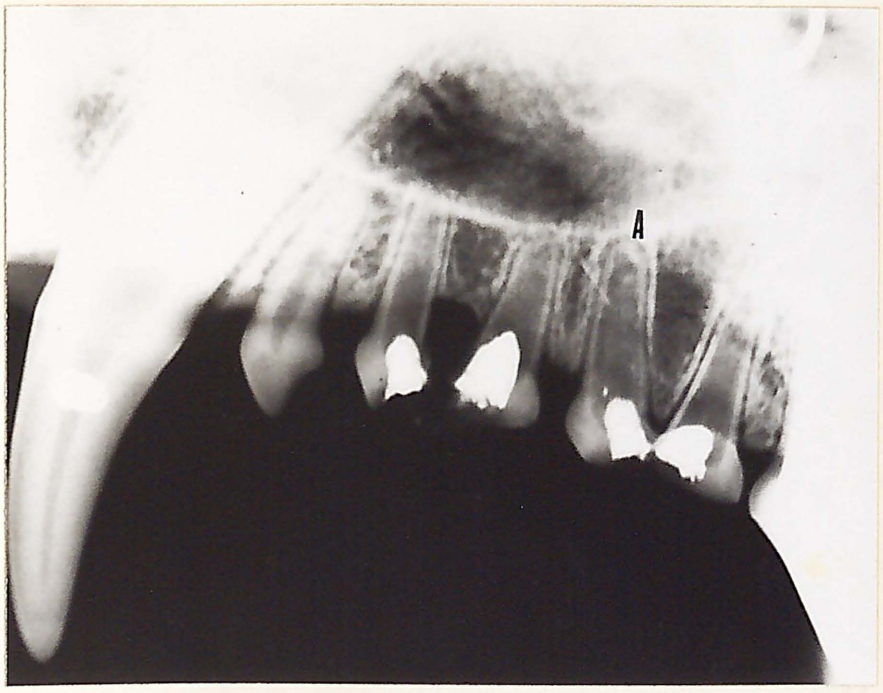
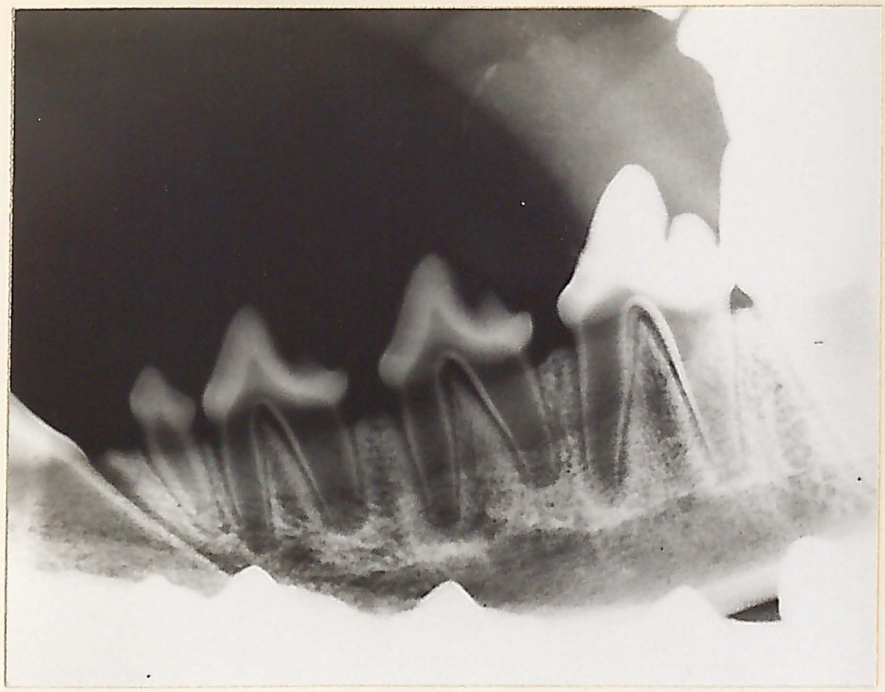

A

Figure 2a: Preoperative mandibular lateral jaw radiograph of dog. Note the wide-open apical foramina.

Figure 2b: Four months postoperative radiograph of same area as Figure 2a. The four canals of the second and third premolars were filled with a paste of calcium hydroxide and distilled water.

AC. Apparent apical closure

C. Granuloma



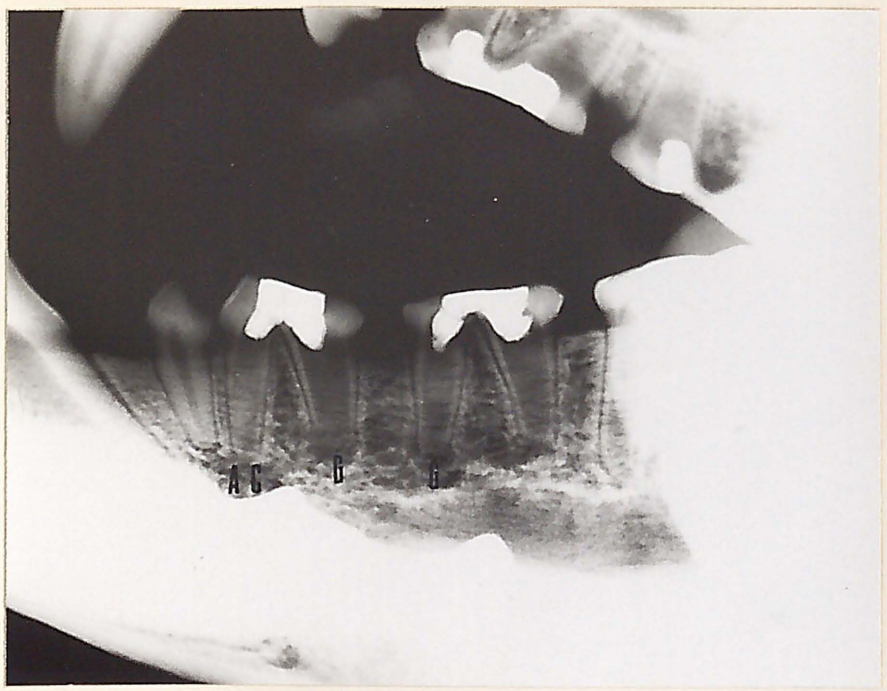

AC
G
G

Figure 3: Untreated maxillary premolar of dog.

A. Alveolar bone

C. Cementum

D. Dentin

F. Foramina

P. Pulp

Hematoxylin and eosin stain

Original magnification, x 2


A
F
C
D
P

Figure 3a: Higher magnification of apical portion of Figure 3. Note the multiple apical foramina.

C. Cementum

D. Dentin

DC. Dento-cementum junction

Hematoxylin and eosin stain

Original magnification, x 100

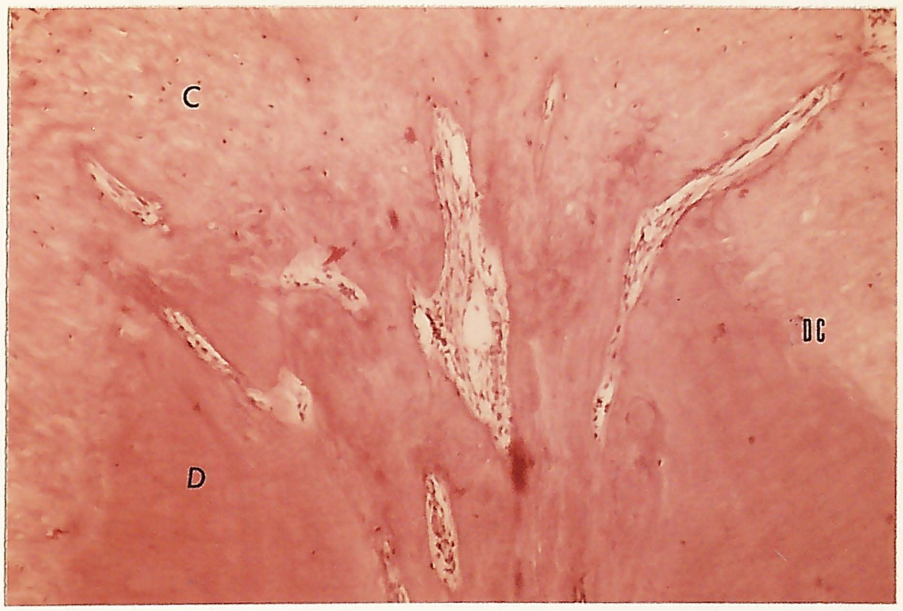
Figure 3b: Fluorescent photomicrograph of similar area to that of Figure 3a. Note the orange fluorescent marking of the Procion dye.

AC. Apical canal

A. Artifact

F. Fluorescent Procion marking

Original magnification, x 100


C
DC
D

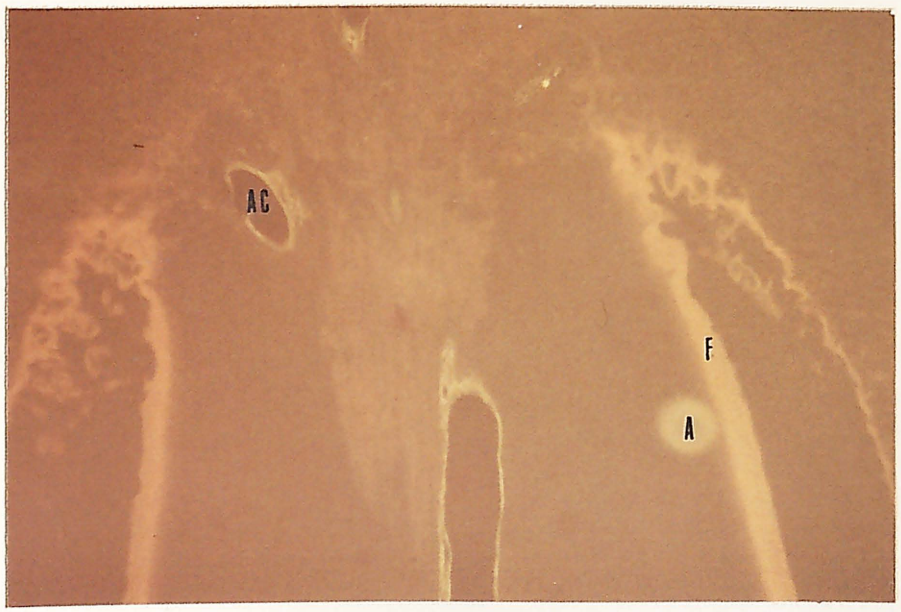

AC
F
A

Figure 4: Hertwig's epithelial root sheath. This dog's tooth, exposed to the oral fluids for one week, has normal pulp in the apical third of the root canal.

Hematoxylin and eosin stain

Original magnification, x 40

Figure 4a: Higher magnification of the root apex.

H. Hertwig's epithelial root sheath

O. Odontoblasts

P. Pulp

Hematoxylin and eosin stain

Original magnification, x 450

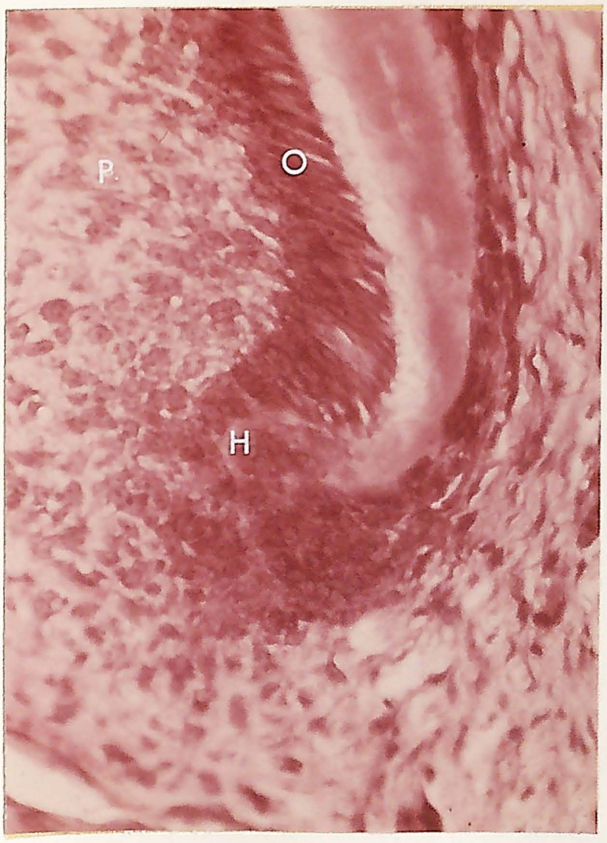

P
O
H

Figure 5: Infected tooth. This tooth had the pulp exposed to oral fluids for one week after laceration of the pulp.

A. Abscess

DS. Dental sac

C. Cementum

Hematoxylin and eosin stain

Original magnification, x 40


A
C
DS

Figure 5a: Apical periodontitis. This is a higher magnification of the apex of Figure 5. Note the inflammatory cells.

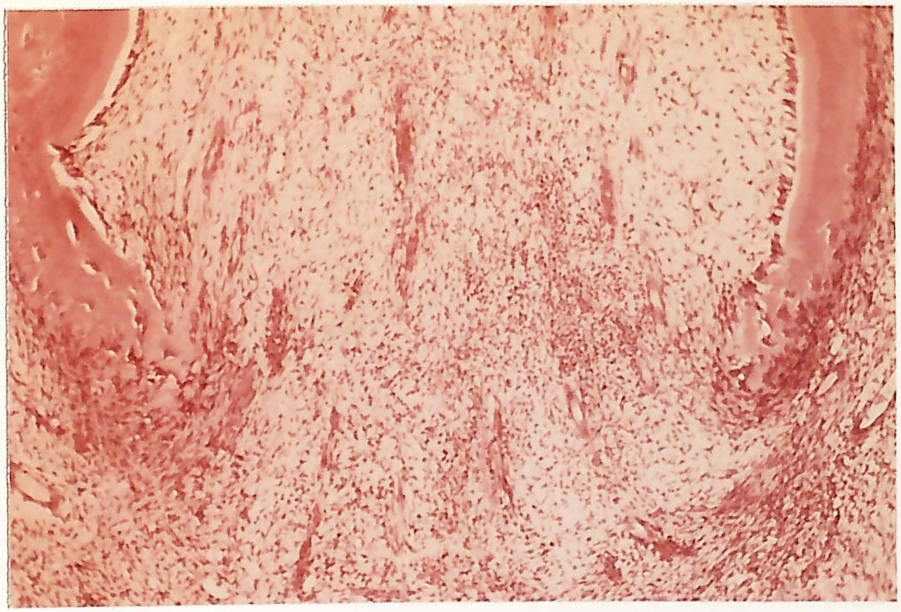
Hematoxylin and eosin stain

Original magnification, x 100

Figure 5b: Fluorescent photomicrograph of same area as Figure 5a. Note that a small amount of dentin has been formed since the Procion was injected.

FP. Fluorescent Procion markings

Original magnification, x 100



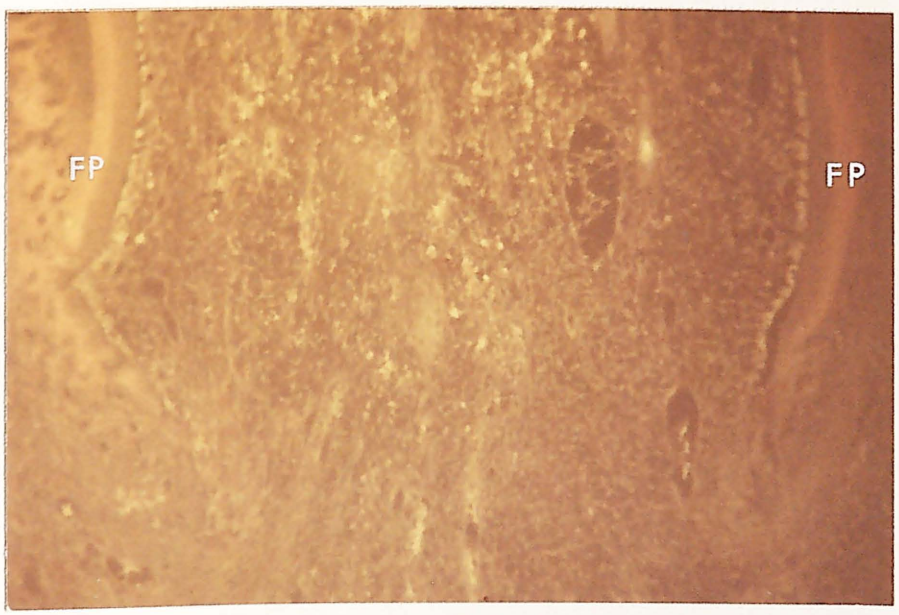

FP
FP

Figure 5c: Higher magnification of periapical tissues of Figure 5a. Note the inflammatory cells, predominantly lymphocytes and plasma cells.

Hematoxylin and eosin stain

Original magnification, x 450

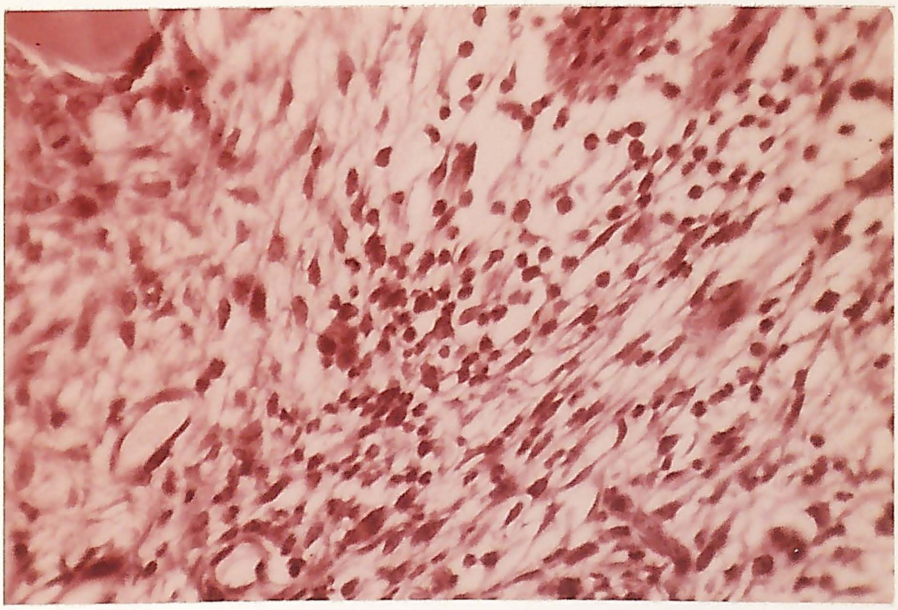


Figure 6: Complete apical closure. Complete closure of the apical foramen by the deposition of calcified tissue resembling cementum has occurred after filling the root canal with a paste of calcium hydroxide and CMCP. Note the deposition of cementum on the periphery of the root apex.

Hematoxylin and eosin stain

Original magnification, x 40

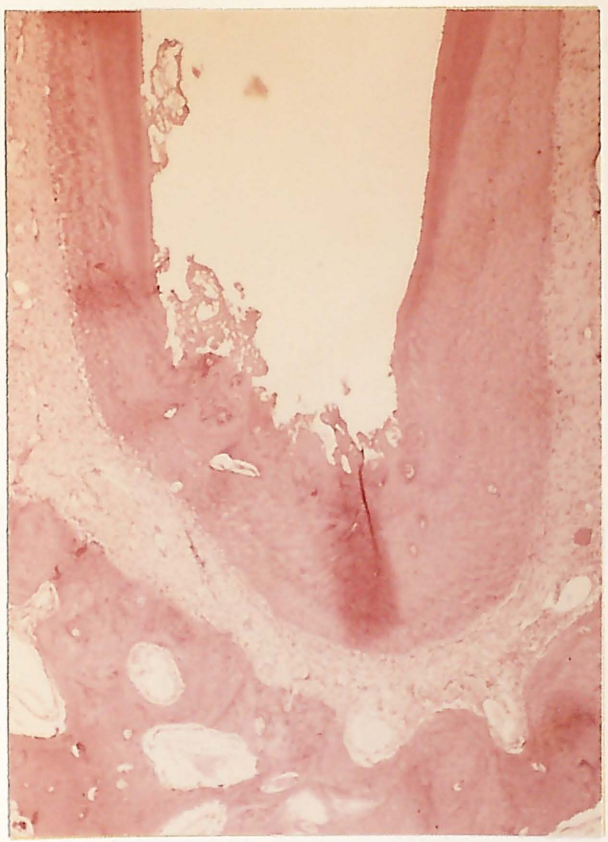
Figure 6a: Higher magnification of calcified tissue shown in Figure 6.

C. Calcified formation

P. Periodontal ligament

Hematoxylin and eosin stain

Original magnification, x 450



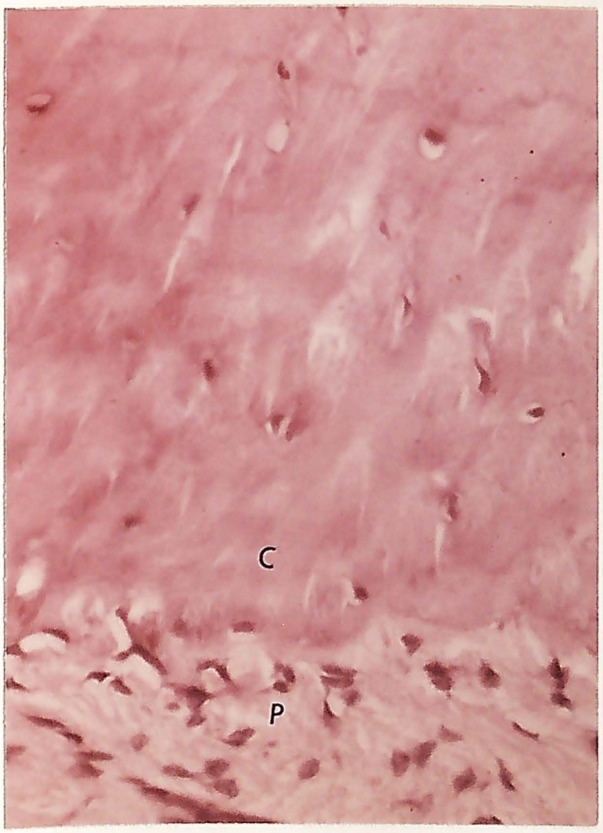

C
P

Figure 7: Complete apical closure. Apical closure has occurred after filling of the root canal with a paste of calcium hydroxide and CMCP. Note the apical formation has occurred around filling material which extended through the apical foramen. The filling material was lost during preparation.

Hematoxylin and eosin stain
Original magnification, x 40

Figure 7a: Higher magnification of the apex of Figure 7. Note the minute foramen communicating with the periodontal ligament.

F. Foramen

FM. Area occupied by filling material

P. Periodontal ligament

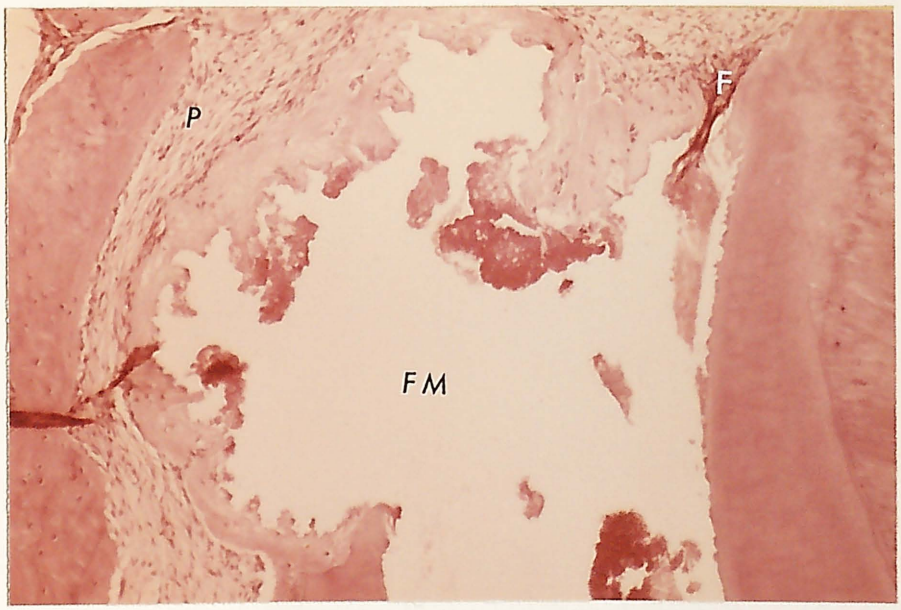
Hematoxylin and eosin stain

Original magnification, x 100

Figure 7b: Fluorescent photomicrograph of same area as Figure 7a. Note that no Procion marking is present in the calcified tissue closing the apex.

FP. Fluorescent Procion marking

Original magnification, x 100


P
F
FM

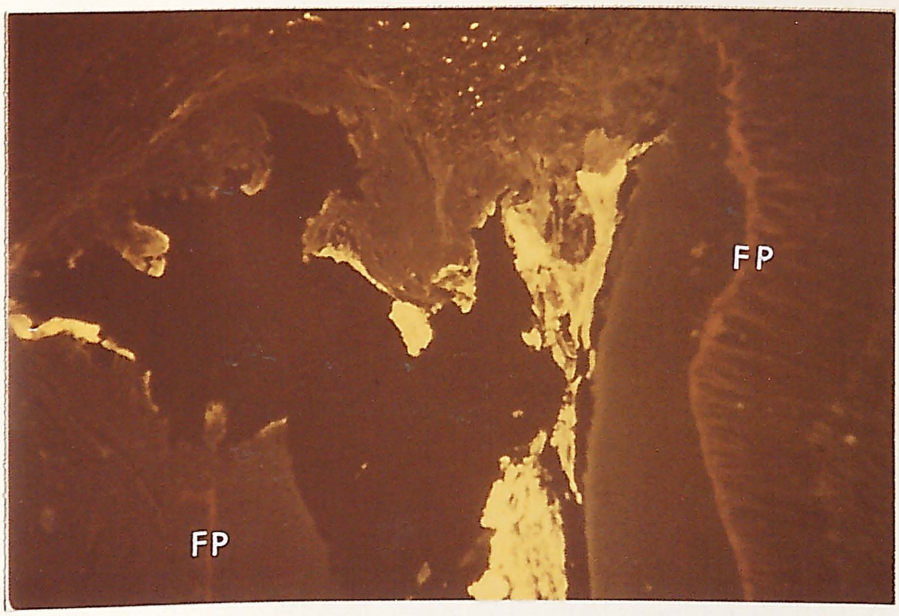

FP
FP

Figure 7c: Higher magnification of apical calcified tissue formation shown in Figure 7a.

C. Calcified formation

FM. Area occupied by filling material

P. Periodontal ligament

Hematoxylin and eosin stain

Original magnification, x 450

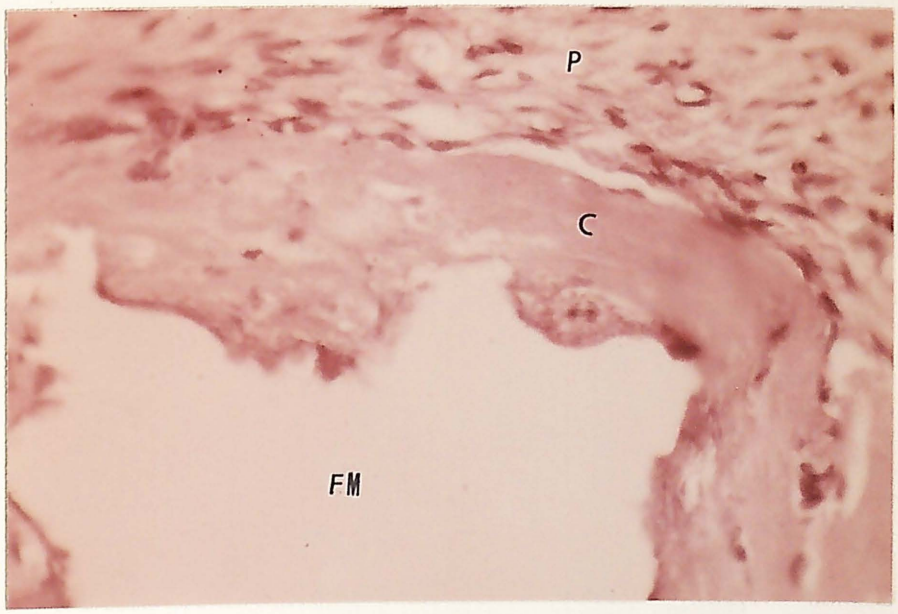

P
C
FM

Figure 8: Blood clot filling apical foramen. A blood clot is present between the periapical tissues and the filling paste of calcium hydroxide and CMCP.

Hematoxylin and eosin stain
Original magnification, x 40

Figure 8a: Higher magnification of the apex of Figure 8. A foreign body reaction with multinucleated giant cells surrounds the blood clot. Note the absence of an inflammatory reaction and the active deposition of bone.

AB. Alveolar bone

B. Blood clot

O. Osteoblasts

P. Periodontal ligament

M. Multinucleated giant cells

Hematoxylin and eosin stain.

Original magnification, x 100

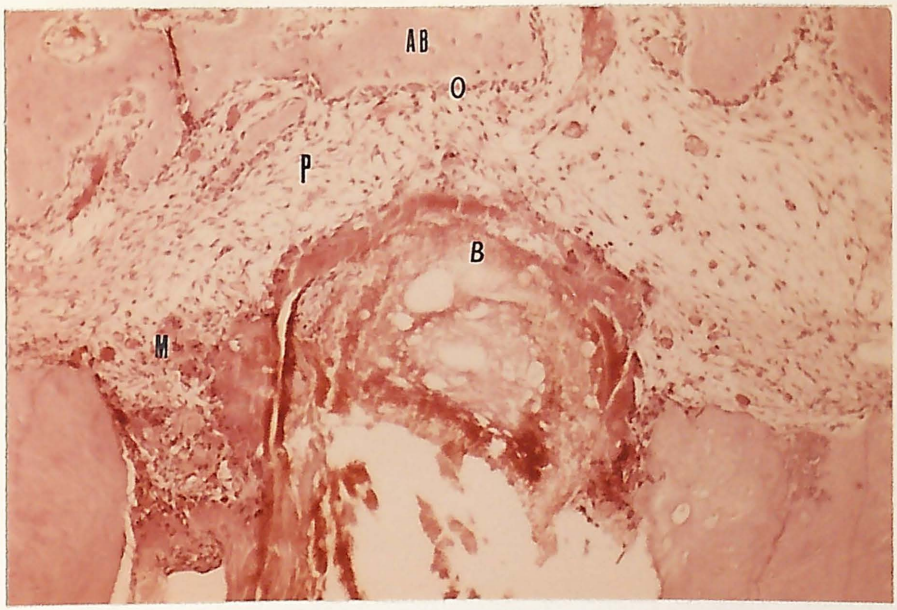
Figure 8b: Higher magnification of Figure 8a. Multinucleated giant cells are seen in the connective tissue surrounding the blood clot.

B. Bloot clot

M. Multinucleated giant cells

Hematoxylin and eosin stain

Original magnification, x 450


AB
O
P
B
M

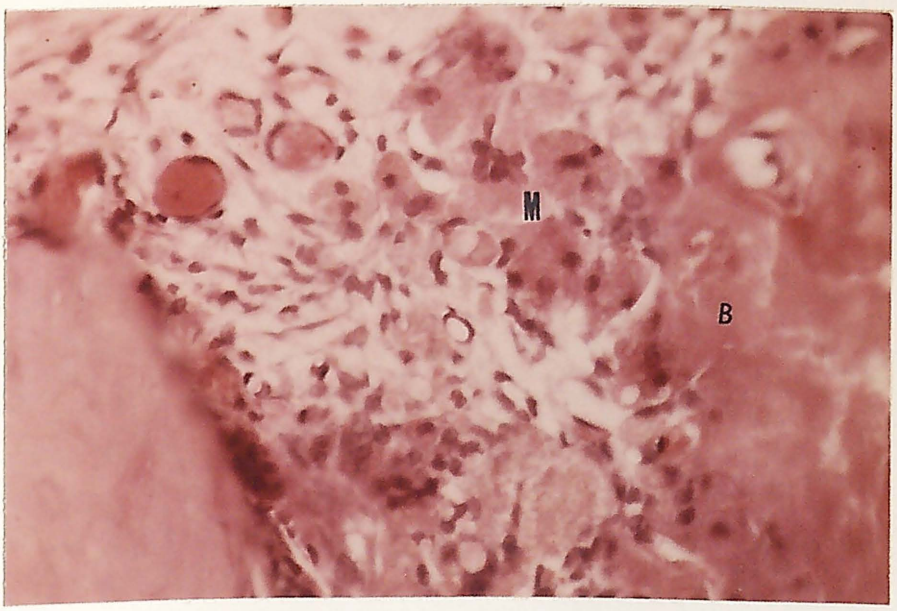

M
B

Figure 9: Moderate inflammatory reaction to root canal filling of calcium hydroxide and CMCP. Note the granuloma formation extending into the root canal.

Hematoxylin and eosin stain

Original magnification, x 40

Figure 10: Complete apical closure. Complete closure of the apical foramen by the deposition of calcified tissue resembling cementum has occurred after filling the root canal with a paste of calcium hydroxide and distilled water. Note the deposition of cementum on the periphery of the root apex.

Hematoxylin and eosin stain
Original magnification, x 40

Figure 10a: Higher magnification of the apex of Figure 10.

FM. Area occupied by filling material

P. Periodontal ligament

Hematoxylin and eosin stain

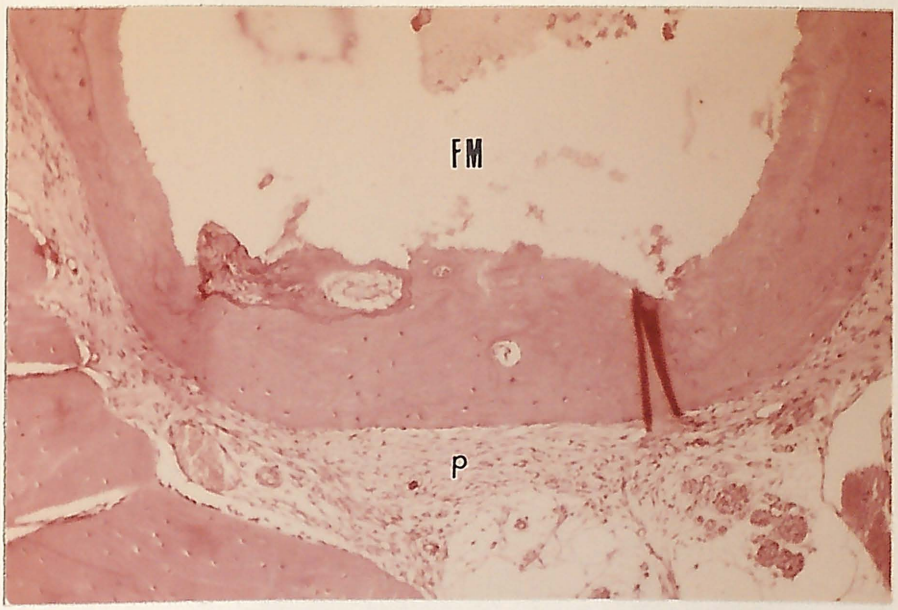
Original magnification, x 100

Figure 10b: Fluorescent photomicrograph of the same area as Figure 10a. Note that no Procion marking is present in the calcified tissue closing the apex.

A. Artifact

FP. Fluorescent Procion marking

Original magnification, x 100


FM
P

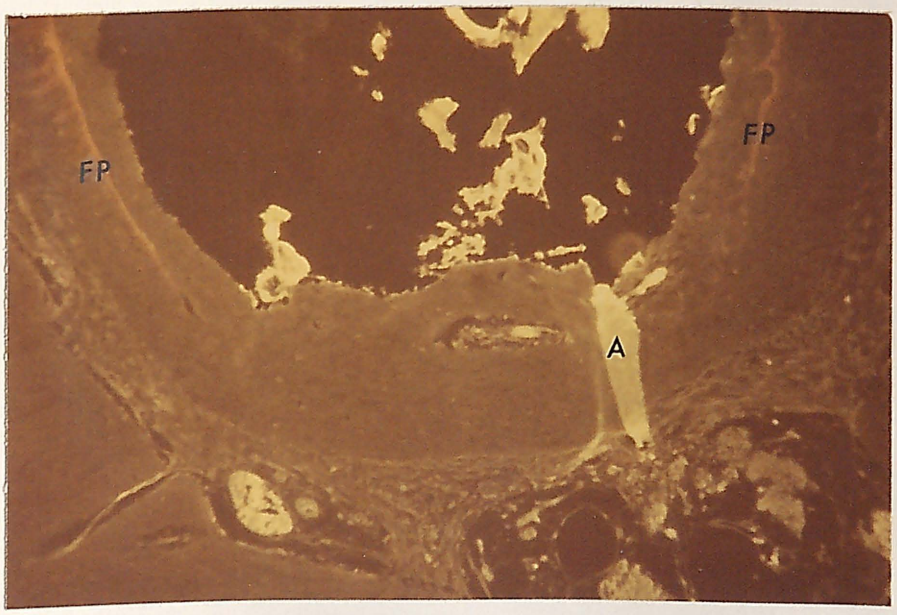

FP
FP
A

Figure 10c: Higher magnification of calcified tissue formation shown in Figure 10a.

C. Calcified formation

FM. Area occupied by filling material

P. Periodontal ligament

Hematoxylin and eosin stain

Original magnification, x 450

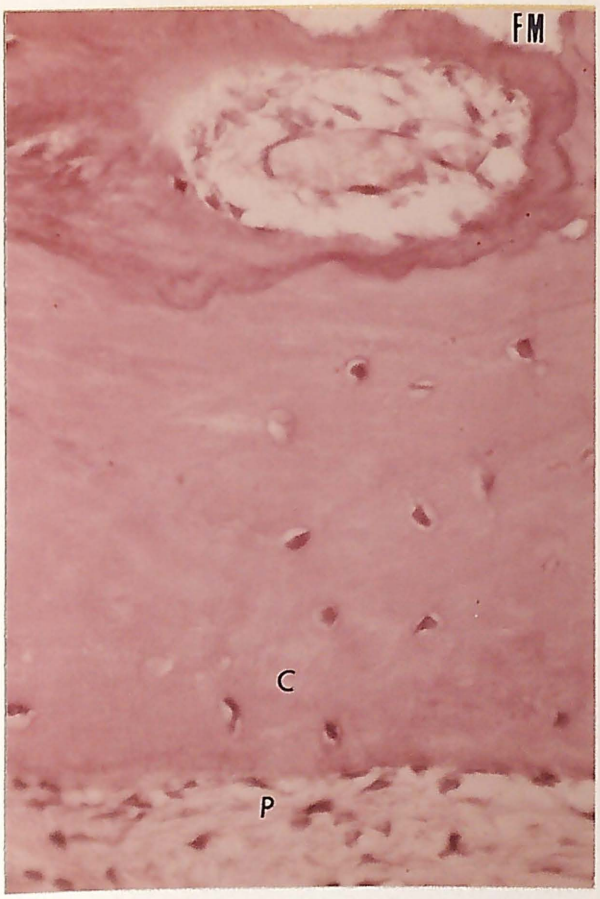

FM
C
P

Figure 11: Slight formation of calcified tissue has occurred after the root canal was filled with calcium hydroxide and distilled water. Note the over-filling of the canal with the paste. The filling material was lost during processing.

Hematoxylin and eosin stain

Original magnification, x 100

Figure 11a: Higher magnification of apical area of Figure 11. Note the active resorption of alveolar bone and small granuloma.

AB. Alveolar bone

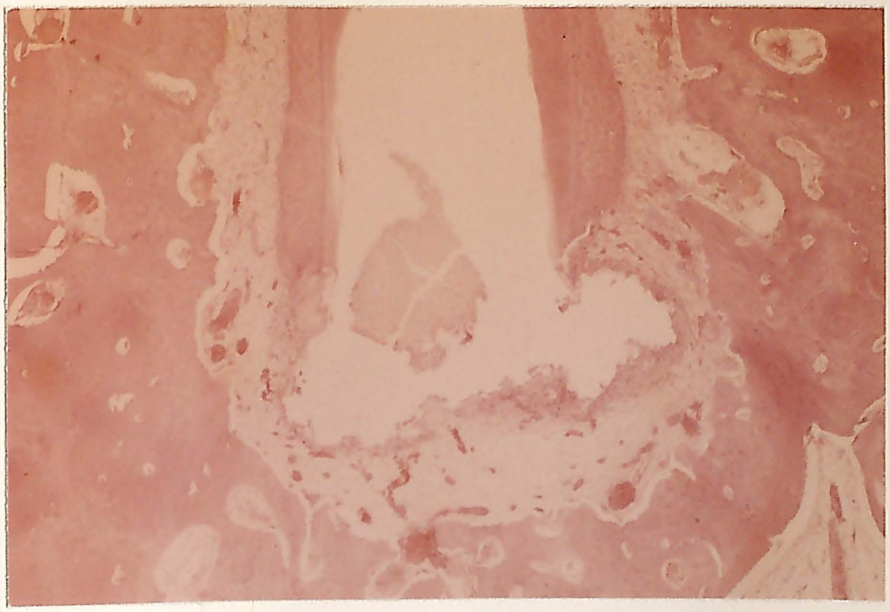
C. Calcified formation

FM. Area occupied by filling material

OC. Osteoclasts

Hematoxylin and eosin stain

Original magnification, x 450



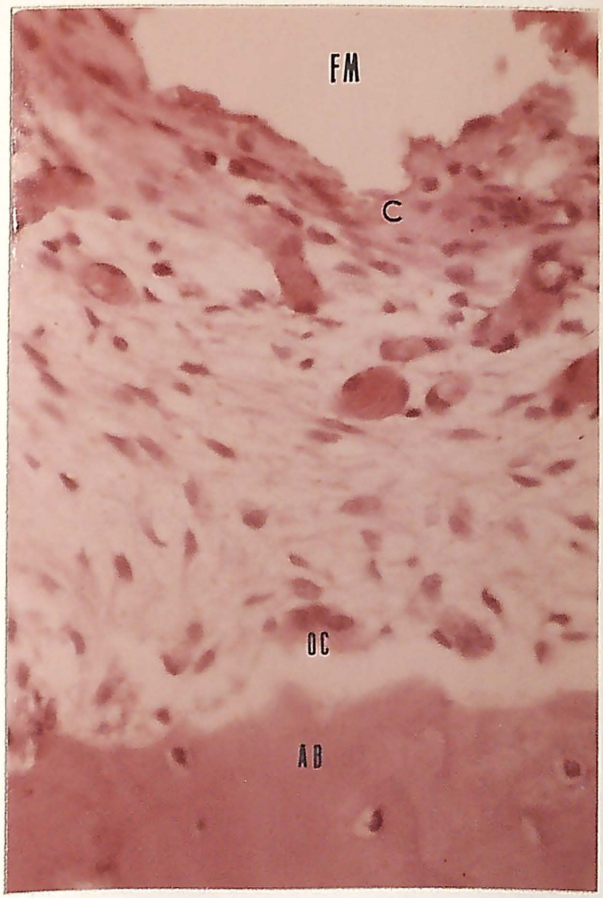

FM
C
OC
AB

Figure 12: Mild inflammatory reaction to calcium hydroxide and distilled water. Note the presence of a small granuloma at the apex of the root. Note the deposition of cementum on the periphery of the root apex.

G. Granuloma

Hematoxylin and eosin stain

Original magnification, x 40

Figure 12a: Fluorescent photomicrograph of Figure 12. One side of the root apex is shown. Note the cementum deposition lateral to the Procion marking.

C. Cementum

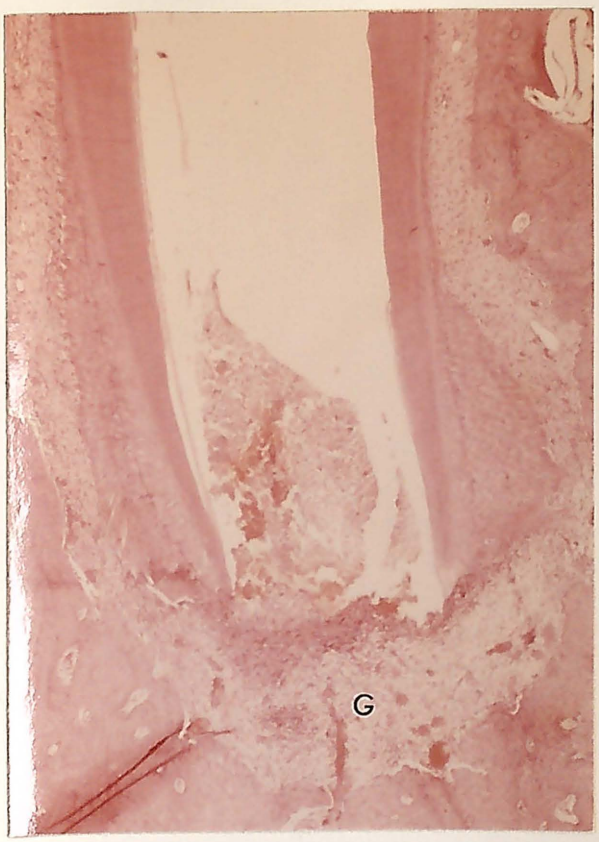
FP. Fluorescent Procion marking

D. Dentin

G. Granuloma

P. Periodontal ligament

Original magnification, x 100


G

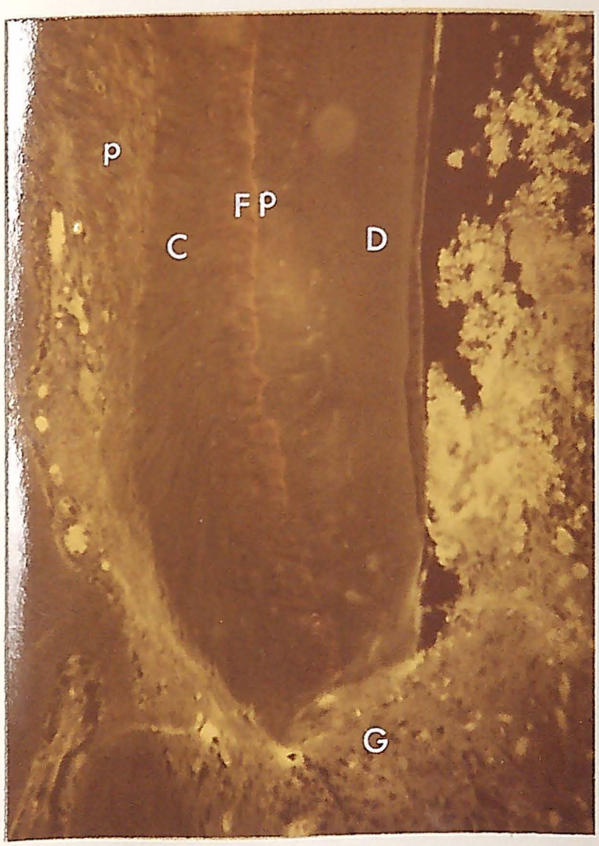

P
FP
C
D
G

Figure 13: Severe reaction to calcium hydroxide and distilled water. A severe reaction with an actively expanding granuloma has occurred after filling the canal with a paste of calcium hydroxide and distilled water. Note the extension of the granuloma into the root canal and the resorption of the root apex.

Hematoxylin and eosin stain

Original magnification, x 40

Figure 13a: Higher magnification of apex of Figure 13. Note the presence of odontoclasts along the border of the resorbing dentin. Many inflammatory cells are present within the granuloma.

D. Dentin

G. Granuloma

OC. Odontoclasts

P. Polymorphonuclear leukocytes

Hematoxylin and eosin stain

Original magnification, x 450

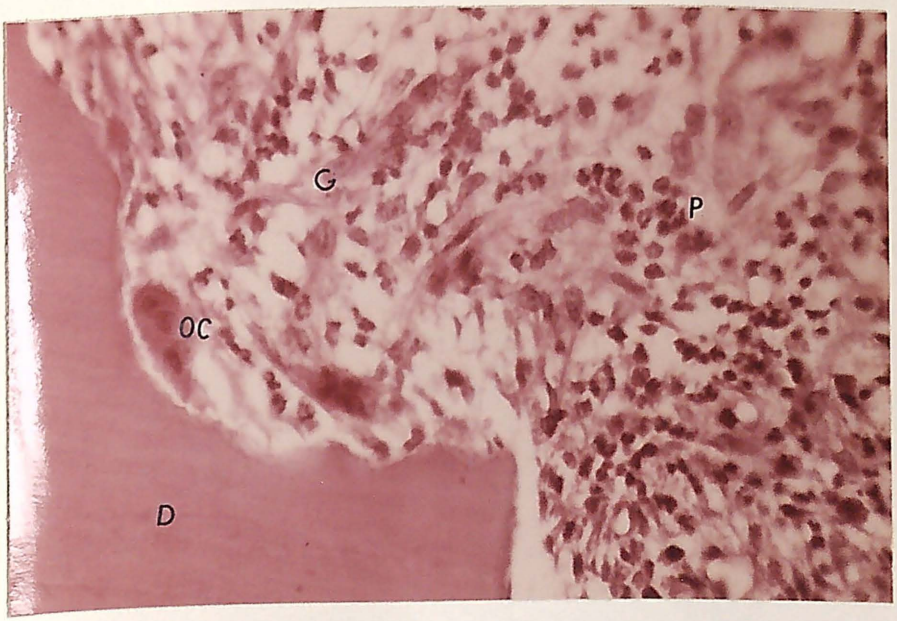

G
P
OC
D

Figure 13b: Fluorescent photomicrograph of apex of Figure 13. One side of the root apex is shown. Note the cementum deposition on the periphery of the root lateral to the Procion marking.

C. Cementum

FP. Fluorescent Procion marking

D. Dentin

G. Granuloma

Original magnification, x 100

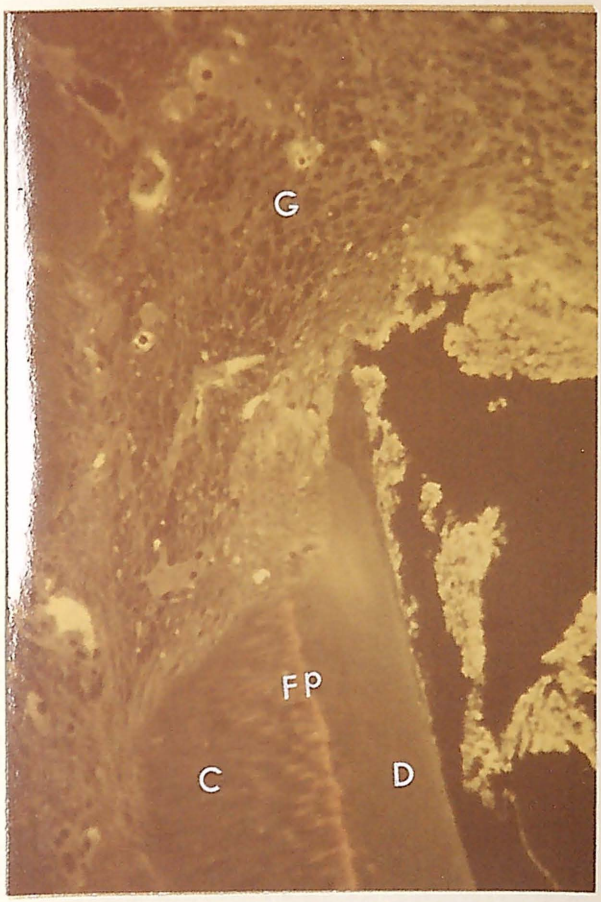

G
FP
C
D

Figure 14: Tooth nearing exfoliation. The pulp of this tooth was exposed to the oral cavity for four months. Note the focal necrosis and severe resorption of the tooth root. A large granuloma surrounds the tooth.

D. Dentin

F. Area of focal necrosis

G. Granuloma

Hematoxylin and eosin stain

Original magnification, x 40


D
F
G

DISCUSSION

Many methods of treating the pulpless permanent tooth having an incompletely developed root with a divergent apex have been advocated in the literature. Until the last decade, the traditional approach has been surgical.[4-6] Techniques reported in recent investigations[9-12, 83-94] have demonstrated radiographically that apical development may continue after the root canals of these teeth are cleaned and a dressing placed in the canals. These techniques have been advocated on the basis of acceptable clinical results and have not been investigated histologically. It was felt by the author that these techniques should be investigated histologically before their acceptance as recommended procedures. The methods of treatment advocated are essentially the same except for the use of different filling materials. Two of the more commonly used filling materials employed to stimulate apical closure were selected for study.

The results of this study show that in the tooth with a necrotic pulp, continued apical development is possible when the necrotic contents are removed and a dressing is placed in the canals. The calcified material formed by the apical connective tissues appeared to be somewhat similar to that induced by Mitchell and Amos[63] Mitchell and Shankwalker,[65] Zawawi[67] and Binnie[74] by implanting calcium hydroxide in the subdermal connective tissue of rats. The location of the material in the dermis caused them to consider it to be osteoid (bone-like) in nature. Under the conditions of this study, wherein, the newly formed material was

intimate with tooth roots and continuous with root cementum, it seems logical to call it cementum. Positive identification of the calcified tissue induced in this study was not possible. Binnie[74] using histo-chemical methods of analysis was unable to identify the nature of the mineral content of the induced calcification. He suggested that the crystal structure could be identified by the use of x-ray diffraction studies.

The formation of apical calcified tissue was found in 31 of the 61 specimens studied histologically. The microscopic appearance was the same in all instances regarding structure. Variation was found only in the amount of calcified tissue formed. Complete, incomplete, and slight closure of the apices, by the formation of calcified tissue, was observed with both types of filling materials.

The findings in this study did not agree with those reported by Seltzer and Associates.[58] They reported no formation of calcified tissue after filling root canals with calcium hydroxide and distilled water. The periapical reaction consistently noted was acute alveolar abscess. However, their longest period of observation was four weeks as compared to four months in this study.

While this study was not designed to compare the effectiveness of the two filling materials, based upon the conditions of this study, the results give the impression that the use of calcium hydroxide and CMCP was superior to the use of calcium hydroxide and distilled water. The formation of apical calcified tissue after treatment was found in 55 per cent of those specimens in

which calcium hydroxide and CMCP were utilized as the filling material. When using calcium hydroxide and distilled water as the filling material, apical calcified tissue was formed in 42 per cent of the specimens. Complete closure of the apical foramen was observed in 29 per cent of the roots filled with calcium hydroxide and CMCP as compared to 11 per cent of those filled with calcium hydroxide and distilled water.

Numerous studies[33-38] have shown CMCP to be an effective anti-bacterial agent. Based on the conditions of this study, the results give the impression that the addition of CMCP to the filling paste is beneficial in reducing inflammation. The presence of inflammation of various types was noted in 79 per cent of those specimens treated with calcium hydroxide and distilled water. Inflammation occurred in only 48 per cent of those treated with calcium hydroxide and CMCP. This study did not include an examination for the presence of microorganisms. This is an area for future study. Utilization of the Brown and Brenn staining technique[102] would be useful in determining the presence of microorganisms.

The mixture of calcium hydroxide and CMCP forms a paste which sets into a hard mass. The mixture of calcium hydroxide and distilled water will not set into a hard mass, and upon dehydration, the paste becomes a powder again. This would allow accumulation of fluids within the canal. It is postulated that the apex of the treated tooth would be better sealed with a material which

forms a hard mass. In those canals which were filled with calcium hydroxide and distilled water, less inflammatory response was obtained when the canals were overfilled. It is speculated that overfilling creates a better seal of the apex and thus a better result.

The manner in which apical closure occurred appeared to be the same regardless of the filling material utilized. The same was true for those apices with incomplete closure. With both complete and incomplete apical closure, the calcified tissue was continuous with the cementum on the lateral surfaces of the roots. In all instances the apices of the roots had been better designed and would have permitted the placement of a routine endodontic filling of gutta-percha as advocated by Cooke and Rowbotham,[10] Frank[9] and Kaiser.[86] Frank[9, 93] has suggested that althought the resorbable seal is adequate to reduce the canal space and contaminants, it should be replaced with a permanent seal to prevent the possible recurrence of periapical pathosis. This procedure seems advisable since it was demonstrated that communications exist between the root canal and the periapical tissue. The resorption of the calcium hydroxide paste in areas of incomplete closure could lead to a failure in the seal of the apex.

Other investigators[46, 98, 100, 101] have reported calcified tissue formation in the presence of inflammation. In this study, the formation of calcified tissue was noted in seven specimens having mild inflammation. The formation was slight in six of

these seven instances. Complete closure was not noted in the presence of inflammation. The chi-square test was used to detect the association between inflammation and apical closure. For the calcium hydroxide and CMCP treated group, the observed chi-square with two degrees of freedom was highly significant ($P < .001$). For the calcium hydroxide and distilled water treated group, the observed chi-square with one degree of freedom was significant ($P < .005$). The coefficients of contingency were calculated to be 0.62 and 0.55, respectively, showing a significant relationship between the two variables.

The presence of blood clot containing red blood cells and fibrin was noted in 20 of the specimens. Varying quantities were noted between the filling material and the connective tissues at the apices. This finding was present with both filling materials. In the specimens demonstrating formation of calcified tissue and containing clot, the latter appeared to be interfering with the complete closure of the apical foramina. Foreign body reactions with multinucleated giant cells were found surrounding the areas of clot on the connective tissue sides. As no reason could be found for the recent occurrence of hemorrhage in the areas, it was assumed that the blood clot had been present from the time of treatment. Foreign body reaction to blood clot four months after treatment led the author to speculate that the clot had been altered chemically by the action of the drugs. It is postulated that the clot was chemically fixed by the action of the calcium

hydroxide since this was the only material common to both filling pastes. To the knowledge of the author, there has been no report of this type reaction to calcium hydroxide. It seems reasonable to assume that the clots would have been eventually phagocytized. If observed over a longer period of time, there would probably have been deposition of calcified tissue in the areas occupied by blood clot. It is possible that complete closure would have been observed in all specimens which showed the deposition of calcified tissue if the study period had been longer.

The four types of apical closure described by Frank[9, 93] were not observed in this study. Two types of closure were observed. The first of these was the formation of a calcified bridge across the existing apex (Figures 6 and 10). The second type was formation of a calcified bridge over an excess of filling material which had been forced through the apical foramen by overfilling (Figures 7 and 7a). In both types, the calcified tissues were continuous with the cementum on the lateral surfaces of the roots.

The observations of previous studies[9-12, 62, 80-94] of continued apical development in pulpless teeth have been radiographic. Only Matsumiya and his coworkers[56] have presented any histologic evidence of their studies. Spedding, Mitchell and McDonald[98] have shown that the use of radiographic interpretation of calcified repair or bridging of successful pulp therapy is faulty and misleading. This observation emphasizes the need for histologic evaluations when determining the value of procedures used in

clinical dentistry.

Unlike human teeth,[28, 99] the full lengths of the roots of the dogs' permanent teeth were established when the teeth erupted into the oral cavity. This was shown by the fact that only one of 10 control teeth in this study displayed the presence of Hertwig's epithelial root sheath. Both human and dogs' teeth have divergent, blunderbuss apices at the time of eruption. However, in humans, root length of the permanent teeth is not completed until two to four years after a tooth emerges into the oral cavity.[28, 99] Spedding and his associates[98] have demonstrated that the development of the teeth of Rhesus monkeys is very similar to that of the human in reference to Hertwig's root sheath. The results of this study might possibly have been more meaningful had the experiments been conducted on monkeys.

Other investigators[9, 10, 12, 93] have speculated that Hertwig's epithelial root sheath may remain intact during infection. Once the infection has been removed, they postulated that the sheath resumed its normal function. No evidence was found in this study to prove or disprove this speculation. The possibility exists that the four types of continued apical development reported by Frank[9, 93] are seen as a result of the presence and function of Hertwig's sheath. Spedding and his associates[98] have demonstrated the presence of Hertwig's epithelial root sheath in the roots of monkeys' teeth erupted into the oral cavity. This is an area for future study which should be investigated using the monkey as an

experimental animal.

All teeth treated in this study demonstrated the formation of cellular cementum on the lateral surfaces of the apical thirds of the roots. The cementum was formed irrespective of the presence, absence, or extent of inflammation present in the periapical tissues. It was demonstrated in the sections of the untreated teeth that this deposition of cementum occurs normally in the dog's tooth as it matures. When radiographs of treated teeth were evaluated, the super-positioning of the cementum over the apical end of the tooth appeared as an apical closure. Therefore, analyses of radiographs of dogs to determine apical closure are not always accurate.

Many of the failures as related to apical closure can be attributed to the difficulty of completely cleaning the root canals of the teeth. Many of the specimens in which apical closure did not occur and in which an inflammatory reaction was present had debris in the root canals. The tooth with an apically divergent root canal is much more difficult to thoroughly clean than the mature tooth with a root canal which becomes increasingly smaller as the apex is approached. In the tooth with a divergent apex, the coronal half of the root canal is smaller than the apical half of the root canal. Therefore, an instrument must be utilized which is smaller than the root canal space. Utilizing this type instrument is much less effective mechanically than using an instrument which contacts the walls of the root canal on all sides.

In this study, the filling material was placed in the root canals immediately after cleansing of the canals. This technique has been suggested by Frank[9, 93] and Natkin.[86] The suggested technique of Kaiser[86] and Friend[60] is to treat the tooth several times. A negative culture is obtained before the canal is filled. The latter technique seems advisable since this would allow more opportunity for complete cleansing of the canals before filling. It is unlikely that a negative culture could be obtained unless the canal was free of gross debris.

A consistent finding in the study was the absence of cyst formation. No proliferation of epithelium was seen in any of the sections.

The resistance of the dog's pulp to infection was demonstrated by the difficulty of inducing necrosis in this study. The pulps were grossly exposed to the oral fluids for one week after instruments contaminated with saliva had been inserted to the apices. Yet, the pulps in four of the 10 control teeth appeared relatively normal in the apical one-thirds while that in the coronal one-thirds was necrotic. During the cleansing of the canals of the treated teeth, pus formation associated with a foul odor was noted in many of the canals. However, in some of the teeth gross hemorrhage was induced during the instrumentation of the canals.

Procion dye has been shown to be an excellent <u>in vivo</u> hard tissue marker.[95, 96] The results found in this study agree with this finding. The dosage of 100 milligrams per kilogram of body

weight as suggested by Tomich[96] proved satisfactory for marking the dental tissues. The use of Procion dye for determination of calcified formation after treatment proved to be effective in this study. The author recommends the use of the dye in future studies of induced calcification.

SUMMARY AND CONCLUSIONS

The purpose of this study was to histologically investigate a clinical procedure which had been recommended as treatment for the pulpless permanent tooth with an incompletely developed root. The pulps of selected erupted developing permanent teeth on a litter of six mongrel dogs were exposed to the oral cavity for one week. Procion red vital dye was injected to demonstrate the formation of calcified tissue after treatment. One dog was sacrificed at the end of one week to serve as a control. The root canals of the teeth in the remaining dogs were mechanically cleaned with endodontic files and frequent irrigation. The canals were filled with two different root canal pastes. These pastes consisted of: (1) calcium hydroxide mixed with camphorated parachlorophenol (CMCP), and (2) calcium hydroxide mixed with distilled water. Five teeth were unoperated. The development of these teeth and the periapical tissues were compared to the corresponding tissues of the treated teeth. The observation period was four months. Decalcified semi-serial histologic sections were made through the teeth and periapical tissues. The sections were stained with hematoxylin and eosin. Alternate sections were left unstained for examination with fluorescent microscopy.

The results of this study show that in the pulpless permanent tooth with an incompletely developed root, continued apical development is possible when the necrotic contents are removed and a dressing placed in the root canal. A calcified tissue resembling cementum was formed at the apices in 31 of 61 treated

root canals. The formation of the calcified tissue, after treatment, was observed in 55 per cent of the specimens treated with calcium hydroxide and CMCP. A complete closure of the apical foramen was present in 29 per cent of these specimens. When using calcium hydroxide and distilled water as the filling paste, the deposition of calcified tissue was present in 42 per cent of the specimens. Complete apical closure was noted in 11 per cent of these specimens.

Although this study was not designed to compare the effectiveness of the two filling pastes, based on the conditions of the study, the results give the impression that the use of calcium hydroxide and CMCP was superior to the use of calcium hydroxide and distilled water.

The presence of an inflammatory reaction was noted in 79 per cent of the specimens treated with calcium hydroxide and distilled water, as compared to 48 per cent of those treated with calcium hydroxide and CMCP. Based on the conditions of this study, the results give the impression that the addition of CMCP to the filling paste is beneficial in reducing inflammation.

The presence of blood clot which apparently had been chemically fixed by the filling material was noted in 20 of the specimens. Foreign body reactions with many multinucleated giant cells were noted surrounding the areas of hemorrhage. The presence of the clot appeared to interfere with the formation of calcified tissue formation.

Differences in the development of the roots of permanent teeth of humans and dogs were discussed. Unlike humans, the full lengths of the roots of dogs' teeth are established at the time of eruption. Hertwig's epithelial root sheath was displayed in only one of 10 control teeth. Because of these differences no evidence was found in this study related to the role of Hertwig's sheath in continued apical development.

The presence of debris in the root canals was noted in many of the teeth which failed to demonstrate apical formation of calcified material. Many of these failures appeared to be related to an inability to thoroughly cleanse the root canals.

Procion dye has been shown to be an excellent in vivo hard tissue marker. The dosage of 100 mg. per kg. proved satisfactory for marking dental tissues. The dye was shown to be effective in determining calcified formation after treatment of the teeth. It is recommended that this dye be used in future investigations of induced calcification.

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ABSTRACT

Continued Apical Development of Pulpless Permanent Teeth Following Endodontic Therapy

by

Joe Henderson Camp

This was a histologic study of continued apical development in the pulpless permanent teeth of dogs. The vital pulps were exposed to the oral fluids for one week. The root canals were filled with either calcium hydroxide and camphorated parachlorophenol (CMCP) or calcium hydroxide and distilled water. A vital dye, Procion red was injected to demonstrate the formation of calcified tissue. After four months, the animals were sacrificed. Decalcified semi-serial sections were studied.

In 31 of 61 pulpless permanent teeth, with incompletely developed roots, continued apical development occurred. Apical calcified tissue resembling cementum was observed in 55 per cent of the specimens treated with calcium hydroxide and CMCP and in 42 per cent of those, with calcium hydroxide and distilled water. Complete closure of the apical foramen was observed in 29 and 11 per cent of the specimens respectively.

Inflammation of the periapical tissues was present in 48 per cent of the calcium hydroxide and CMCP group and in 79 per cent of the other group. A significant association was found between the degree of inflammation and apical closure, ($P < .001$) for the calcium hydroxide-CMCP group, ($P \leq .005$) for the calcium hydroxide-distilled water group.

The results suggest that calcium hydroxide and CMCP was superior to calcium hydroxide and distilled water and that the addition of CMCP to the paste reduced inflammation. Procion dye was shown to be an effective *in vivo* dental hard tissue marker.